PARASITOLOGY

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(Founded by G. H. F. Nuttall, F.R.S.)

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P. A. Buxton.

PATRICK ALFRED BUXTON

1892–1955

Patrick Alfred Buxton was born in Hyde Park Street, Paddington, on 24 March 1892, and died at his home in Gerrards Cross, Bucks, on 13 December 1955. His father was Alfred Fowell Buxton, a busy banker and one time chairman of the London County Council, his mother was Violet Jex-Blake.

His father's family was part of that immense group of Buxtons, Gurneys, Barclays, Hammonds, and Hoares, many of them resident in Norfolk and many of them Quakers, whose names appear in the annals of business in this country, in banking, in brewing, in the wool trade and so on, over many centuries. This reputation for acumen in business was often combined with a most practical interest in philanthropy and in political or social reform. One may recall the active part played by Sir Thomas Fowell Buxton in association with Wilberforce in the abolition of the slave trade; and the part played by his sister-in-law Elizabeth Fry in prison reform, and of his grandson Sydney Charles Buxton who sponsored the Wild Birds Protection Act of 1902 and did much to improve conditions of employment. Some branches of the group, for example Hanbury the gardener and Francis Galton, were prominent in science—but to trace a connexion between Patrick Buxton and Galton one would have to go back five or six generations to David Barclay, son of Robert Barclay 'the Quaker apologist'. Among the distinctions of these ramifying families literature is hardly represented and the arts not at all—most were country gentlemen, good naturalists, and strongly averse to things academic.

His mother's family was not numerous; they were mostly farmers and landowners in West Norfolk. His grandfather on his mother's side, Thomas William Jex-Blake was a classical scholar, Headmaster of Cheltenham and Rugby schools and then dean of Wells—with a strong antipathy to biology and the sciences in general. He married a Cordery and had a large family, several of them very able, among whom one might mention Kathleen Jex-Blake, classical scholar and Mistress of Girton, Cambridge, Henrietta Jex-Blake, Principal of Lady Margaret Hall, Oxford, and Arthur Jex-Blake, an able scholarly medical man.

There were three children in the family of Alfred Fowell Buxton, of whom Patrick was the eldest. His brother Denis Alfred Jex Buxton (*b.* 1895), also a keen naturalist, followed a career in the City; his sister Violet Elizabeth (*b.* 1900) remained at home and died a few years ago. Until Patrick was eight years old the family lived in London—in the days when it was still possible for a small boy to ride his pony from the Edgware Road across to the Marble Arch. But there were long summer holidays in the countryside of Hertfordshire. The family then moved to completely rural surroundings, several miles from the railway, at Chigwell in Essex.

Outside his busy life as a banker, Alfred Fowell Buxton was fond of gardens, shooting, birds and moths and this was a strong influence on the small boy. There

was a family tradition of knowing plants, animals and birds and of collecting them. The young naturalists were most industrious, preserving and labelling and 'making collections'; there was a family Natural History Journal (an old Quaker custom) and at rare intervals they would record the catching of a water shrew, seeing a rare bird or finding a flower at an unusual date—but no one guided them further to think about their specimens or use them.

Patrick Buxton was educated at home until the age of ten. By that time he had already started on Greek—and had been imbued with a strong and lasting prejudice against the classical languages. This was followed by three years at a preparatory school with more classics and little profit, and from 1905 to 1911 he was at Rugby. Under the strong Jex-Blake influence he was kept on the classical side, which bored him to the extent that he was nearly superannuated. Indeed, looked at on the surface his school days were not a success. Short sighted, untidy in appearance, a near failure at work and with no aptitude for games, he became something of a butt—the sort of boy whose subsequent distinction his school fellows find hard to credit.

There was one bright spot. This strange youth was allowed to neglect organized games for the pursuit of natural history; he ran the school Natural History Society and published many local observations in their annual report. Of formal teaching of biology there was none, and scholarships and prizes did not come Patrick Buxton's way.

In October 1911, again in accordance with a strong family tradition, he went up to Trinity College, Cambridge. His tutor was the physiologist Walter Fletcher, to whom he explained that he was a complete failure at academic work and proposed to take a Pass Degree in biological sciences. But Fletcher insisted that he should at least try for a Tripos and that if that proved impossible they could think about the Pass Degree after a year.

That was an encouraging start; for Buxton quickly discovered that he had been completely misled about his abilities—that he could actually do intellectual work in biology as well as the next man; and in 1914 he obtained a 1st class in Part I of the Natural Sciences Tripos with zoology, botany and physiology as his subjects.

About November 1913 Buxton has added medicine to his burdens—under a philanthropic urge that this was of more use to humanity than pure biology. And on the outbreak of war in August 1914 he enlisted as a private in a Field Ambulance, thinking that a medical student should be of some use in such a place. But in January of the following year he was sent back to his medical studies.

In the summer of 1915 he rather rashly sat for Part II of the Natural Sciences Tripos in zoology, for he had been mainly working on his anatomy and physiology for the 2nd M.B. examination, and it gives some indication of the enthusiasm with which Buxton was developing his newly discovered powers, that he not only passed the 2nd M.B. but obtained a 1st class in the Tripos.

In the old Department of Zoology at Cambridge in those days, conditions were so informal that the keen undergraduate could sit about and pick up all the methods that he wanted and learn informally from men a little older than himself in a way

that is less easy in these crowded days. In physiology Buxton owed much to his informal contacts in this way with Keith Lucas, in the days when he and A. V. Hill were doing such brilliant work in such unsavoury surroundings.

In the autumn of 1916 Buxton was elected to a Fellowship at Trinity. This was awarded mainly for a piece of undergraduate research on the brain of the primitive moth *Micropteryx*. The idea of this research was entirely his own and was not very logical: the object was to attempt to throw light on the structure of the insect brain studying the brain in a primitive insect—but *Micropteryx*, though a primitive Lepidopteron, is a member of one of the more recently evolved groups of insects. It must have been difficult work to assess, remarkable chiefly for the extremely difficult conditions, in camp and so on, under which it was carried out; but Stanley Gardiner, who was one of the assessors, and who must have started without any knowledge of the subject, doubtless recognized the potentialities of the author.

Buxton proceeded to St George's Hospital and qualified in medicine (M.R.C.S., L.R.C.P.) in 1917. He at once took up a commission in the Royal Army Medical Corps, and was posted to Mesopotamia and North West Persia. Often there was very little to do and, in consequence, plenty of opportunity for the enterprising naturalist to collect great numbers of insects, bird skins and the like. The censor raised no objection to the provision of adequate data and most of the labelled specimens reached home safely. All this material, with much else obtained by other collectors, formed the basis of a survey of the fauna of Iraq published by the Bombay Natural History Society. It also provided a fine training in the natural history of desert animals, although, being constantly on the move, he was unable to do any serious biology. This lasted until the end of 1919.

During 1920 and 1921 Buxton was back in England, resident mostly in Cambridge where he was working on the speciation of the itch mites *Sarcoptes* in Nuttall's laboratory. He also took the course in tropical medicine in London—where, according to tradition, he was chiefly noted for his habit of arriving late and reading the newspaper during lectures—characteristics which in later times he found impossible to tolerate in students. Which goes to show how our point of view changes with the years!

In March 1921 Buxton accepted the post of entomologist in the Medical Department in Palestine. Here he remained for two and a half years, devoting himself on the one hand to the survey of malaria and other parasitic diseases (sound but unenlightened work according to his own assessment, for he had no contact with anyone who really knew about malaria) and on the other to the study of desert and semidesert faunas. The output from two years' work was remarkable and culminated in the production of what he himself has called that 'vigorous young man's book' on *Animal Life in Deserts*.

By the summer of 1923 Buxton was beginning to feel that he had been enough in Arab and Persian lands, and that if he was to be a real medical entomologist he ought to see the damp tropics. This opportunity came when he accepted a temporary post under the London School of Tropical Medicine to lead a research expedition to Samoa. The purpose was to continue the Manson tradition and to pursue the

study of filariasis. The plan was somewhat vague: perhaps to control the insect vector, perhaps to do work on its biology. Anyhow, he had plenty of equipment, generous terms and complete independence; so that the vagueness of plan was probably what he would have wished. He chose his own assistant, G. H. E. Hopkins, a careful worker and a good naturalist, and together they spent two years in Samoa.

During that time an immense amount was accomplished. The general biology of *Aedes scutellaris* (*variegatus*), which transmits the infection, was known from the work of Manson-Bahr and others, but they carried out a great many simple experiments in an attempt to analyse the specialized selection of breeding places by the egg-laying female—a line of study which has been much developed since but was new at that time. Buxton also visited the New Hebrides for a few months and made a useful survey of the general incidence of malaria, filariasis and other diseases, and he spent a month touring the Ellice and Tokelau Islands in a mission ship. In this way he was able to study a true sample of Oceania and became familiar with the ways of life of Polynesians and Melanesians in the widely dissimilar types of island. All these surveys and experiments were published in two large memoirs by the London School of Hygiene and Tropical Medicine.

As a side line Buxton and Hopkins collected Samoan insects. Instead of the very restricted fauna that would all go into 'two or three cigar boxes' which some entomologists had foretold, they collected about 1600 insect species, many of them new to science. All were eventually listed or described in a series of memoirs on *The Insect Fauna of Samoa* and published in good style by the British Museum. All this amounted to a substantial achievement which entailed the co-ordination of the work of experts in all parts of the world, and in the result the insects of Samoa were better known than those of nearly all the other island groups in Oceania.

The Pacific trip lasted from the end of 1923 to January 1926. Buxton was beginning to feel that it was about time to settle in England. In January 1917 he had married Muryell Gladys Rice, daughter of the Rev. Hon. William Talbot Rice (the son of a Baron Dynevor—a family of Welsh landowners and coalmine owners) and Marian Gurney. Buxton and his wife were therefore second cousins and doubtless related in other ways, their large connexions having intermarried frequently and over a long run of years. They had gone out to Palestine with one small baby, a son, and returned with two. A daughter was born in Samoa. These simple statements will perhaps serve to convey the extent to which Buxton was indebted to his wife during these rather hectic years. Though not sharing his scientific interests she always provided a home and a background and shared to the full his interest in the places and people around them.

By the time Buxton was back in London in January 1926 he had been appointed head of the Department of Entomology in the new London School of Hygiene and Tropical Medicine, which had absorbed the old London School of Tropical Medicine and was now gathering itself together under Andrew Balfour. The great new building in Gower Street was only on paper, and in an early state. Several departments (bacteriology, epidemiology) had not yet been formed and when formed had to be housed separately, in several parts of London.

The Department of Entomology in the old School had been the creation of Colonel A. C. Alcock—an able and scholarly medical entomologist, much loved and respected, but sadly aged by the time of his retirement. All the departmental letters, many of them unopened, were kept in a soap box. There was not one single piece of graduated glassware, no balance. But, with the large Rockefeller donation, there was plenty of money available for equipment.

At this time Buxton wrote a letter to *Nature* in which he put forward the view that 'real progress will not be made in applied entomology until...we devote time and labour to the study of the fundamentals of insect physiology'. Following this up he persuaded the Board of Management that 'the contribution of the School to medical entomology should be to go deep and to produce a science of insect physiology'—as a basis for the understanding of insecticidal action and for the interpretation of biology in the field. Walter Fletcher, who was now Secretary of the Medical Research Council and a very active member of the Board of Management of the School, strongly supported Buxton in this point of view.

In advertising for an assistant, with a very wide range of desirable qualifications, a special point was made of experience in biochemistry or some side of physiology —a highly original requirement in those days when filling a post in medical entomology. Among the applicants was V. B. Wigglesworth, 'who had had a good academic career, had written half a dozen papers, some jointly with J. B. S. Haldane, was about to qualify in medicine—and was very silent and unimpressive at interview. Against him were middle-aged people, with lots of field experience in anti-malarial work, etc.' Buxton, very young and very definite, strongly supported Wigglesworth at the appointing committee; he was backed up by Walter Fletcher and the appointment was made—although, as Balfour did not hesitate to point out to the successful candidate, it was a very queer one. These facts are perhaps worth putting on record because Buxton always rightly felt that one of his most valuable contributions to entomology was the impetus he had given to the study of insect physiology, and this was his first step. In this way began the happy (though not uncritical) association between the present writer and Patrick Buxton that was to last for nineteen years.

There followed the busy period of planning and equipping the new Department, of building up the teaching courses in medical entomology, and of putting the idea of research in insect physiology into practice. Buxton himself took up the study of the relations of insects to climate, their water and temperature relations. More will be said about this when we come to discuss Buxton's scientific contributions; it was the general field of research that occupied his attention between 1926 and 1939. Other appointments were made, the most notable of which were that of K. Mellanby who joined Buxton with the support of the Medical Research Council to extend the work on water and temperature relations—which he did to very good purpose—and R. P. Hobson whom the Empire Marketing Board financed to study digestion and nutrition in Calliphoridae.

By 1931 Buxton began to feel the need to travel once again. He made a brief trip to Palestine in an attempt to study the microclimate in which the fleas of rodents actually exist in hot dry countries. In 1933, in company with a young

graduate D. J. Lewis, a distant cousin, he undertook a more serious expedition of seven months in Northern Nigeria, where they carried out extensive observations on the climatic conditions in which the several species of tsetse flies develop in this hot dry part of Africa. In the same year he was made Professor in the University of London.

Throughout these years a long series of entomologists from all parts of the tropics visited the laboratory for short periods. And many others, after a period of training in laboratory research, have had distinguished careers as medical entomologists: D. J. Lewis, R. C. Muirhead Thomson, A. Haddow, E. P. Hodgkin, and many more. In medical entomology the most important enterprises were a detailed investigation of the physiology and ecology of the bed-bug, carried out by C. G. Johnson with the support of the Medical Research Council (1935–40), a study of the factors concerned in the spreading of anti-malarial oils by D. R. P. Murray supported jointly by the major oil companies (1936–40), and an analysis of the egg-laying behaviour and biology of *Anopheles minimus* in Assam by R. C. Muirhead Thomson under a grant from the Royal Society.

For a year or two in the late nineteen thirties Buxton had been working on a projected text-book of medical entomology. One chapter was finished, that on the louse. On the outbreak of war in September 1939, thinking, as many did, in terms of the earlier world war and picturing again a stationary warfare in trenches, Buxton decided to devote the main effort of his department to the improvement of practical measures against the louse. The chapter in the book was rapidly expanded to form an up-to-date monograph on the subject and within seven weeks of sending to the press it was published.

Twenty years had gone by since there had been any serious work on the control of lice. The accumulated experience of twenty years in applied entomology was available for application in this field. Buxton appreciated that the great shortcoming of all the de-lousing procedures developed during the 1914–18 war was that they had no lasting effect: immediately the man returned to the trenches he was re-infested. The answer appeared to be a 'lasting' insecticide with which the clothing could be impregnated. There had been considerable advances in insecticides in the course of twenty years and particular attention was given to the organic thiocyanates.

Buxton's methods of conducting this investigation were energetic, unconventional and Buxtonian. They were not very popular with the authorities of the London School of Hygiene. By means of contacts with relieving officers and the keepers of common lodging houses, he built up a clientele of verminous tramps who (for a small consideration) used to flock to the 'louse clinic' in the School. Here they were given new shirts for old, or their shirts were sprayed or dusted with various new chemicals. Meanwhile everyone possible, including most of Buxton's family, were pressed into the service of rearing lice in small containers under their socks or vests to provide the material for controlled experiments in the laboratory.

The unsavoury and unconventional aspects of this enterprise appealed to the showman in Buxton; but it was all most useful work. Only one thing was lacking

—a suitable insecticide. The thiocyanates were effective in killing lice but they were too irritating to the human skin for general use. On the other hand, incorporated in a hair oil, they proved invaluable in the treatment of head lice which were a major problem at this time both among the children evacuated from the large towns and the girls recruited to the Women's Services. He developed a pleated impregnated belt which could be worn beneath the clothing—but this also proved too irritating during sweating—as became only too apparent when a certain Highland regiment carried out an exercise on the Cairngorms wearing the impregnated belts.

By 1942 this work had reached a deadlock. Then we were approached by Dr G. A. Campbell of the Geigy Chemical Company in Manchester (he had been referred to us by Dr F. Tattersfield) with the report that their parent company in Switzerland had produced a new synthetic insecticide which they wished to make available to the western allies. We read the patent specification of 'Gessarol', and were frankly sceptical; for this material, non-irritating and apparently harmless to man, non-volatile and long-lasting, highly toxic to insects, was exactly what Buxton had visualized as the lasting insecticide against the louse. Of course the material had to be tested. A small sample was synthesized at Manchester; a kilogram sample was obtained from Switzerland. Both had the same properties; the material was even more promising than we could have hoped. Almost at the same time the branch of Geigy in the United States brought the new insecticide to the notice of the American authorities. They were equally impressed. Soon afterwards, at a meeting of the Insecticides Research and Development Committee, the Chairman, Professor (later Sir Ian) Heilbron christened the new insecticide 'DDT'. The name was accepted by the Americans and the rest of the story, which marked the beginning of a new era in applied entomology, is well known. Buxton's preparatory work proved very useful in the early exploitation of DDT.

Buxton's interest and energy was by no means confined to the louse problem. On the outbreak of the war he established close relations with the medical departments of the War Office, Admiralty and Air Ministry. He organized series of lectures to nurses and shelter marshals. He persuaded Sir Edward Mellanby as Secretary of the Medical Research Council to set up an Entomological Sub-Committee of their Military Personnel Research Committee, with Buxton as chairman. This proved a useful medium during the early war years for advising the Service Departments on modern methods in medical entomology. It was not so effective as it might have been for it had no means of ensuring that its recommendations were carried out. That defect was later remedied by an ingenious administrative device in a new committee, the Pyrethrum Development Panel (later the Insecticides Research and Development Committee) under the chairmanship of Professor Heilbron. It was characteristic of Buxton that he was not interested in the slightest degree in his personal position in these affairs. He was concerned only in improving the methods of medical entomology in the Services.

Buxton certainly enjoyed his war. It provided all the opportunities he could desire for deploying his unbounded energy. Work was initiated on fumigation against lice in clothing and bedding, on mosquito repellents, on mosquito and

fly sprays, on the control of bed bugs in air-raid shelters, on the control of head lice and other projects. He made trips to the United States as a member of a mission from the Ministry of Production to agree on the sharing of pyrethrum supplies, to Egypt to try out the louse belts (which proved of real value when used among native labour forces in the Eastern Mediterranean), to West Africa to take part in trials of aerial spraying against mosquito larvae. His ambition of making a trip to Russia to demonstrate the new methods of controlling bed bugs and body lice was thwarted only by Madame Maisky (the wife of the Russian Ambassador), who solemnly assured him that neither of these insects existed any longer in Russia.

At the end of the war in 1945 when Buxton had to re-start his department on a peace-time footing, he felt disinclined to go back himself to physiological research. He even suggested more than once in conversation that the study of insect physiology had not yielded quite what he had hoped. But he attracted workers in many sides of medical entomology and his department was never more active. It was at this time that as a member of the Colonial Medical Research Committee (of the Medical Research Council jointly with the Colonial Office) Buxton was invited to carry out two extensive tours in East Africa, from Kenya and Uganda to Zululand and Bechuanaland, to report on the tsetse fly and trypanosomiasis with reference to the organization of research and control. These long journeys occupied much of 1945 and 1946.

The last decade saw the building up of the Department of Entomology as a most active centre of teaching and research. Buxton became increasingly in demand as an adviser and as a member of committees. He had been elected a Fellow in 1943 and served on the Council of the Royal Society for 1951–53. He was appointed a member of the Medical Research Council in 1945 and served on their Committees dealing with Typhus, Malaria and Colonial Medical Research, as well as the Tsetse Fly and Trypanosomiasis Committee of the Colonial Office. He served on the Council of the Royal Society of Tropical Medicine and of the Royal Entomological Society, of which he was President in 1942 and again in 1953–55. He was made a C.M.G. in 1946. In 1949, on the occasion of the jubilee of the Liverpool School of Tropical Medicine, he was one of the recipients of the Mary Kingsley Medal. In 1951 he received a Linnean medal and in 1953 the Gold Medal of the Linnean Society. In 1950 he was elected to Foreign Membership of the Société de Pathologie Exotique. During these latter years, however, Buxton continued to travel: to the Southern Sudan to study tsetse flies, to Canada to see the black-fly and mosquito problems of the far north, to Central America to gain first-hand knowledge of an epidemic of jungle yellow fever, and to Malaya. Indeed, between 1940 and 1954 there was no year in which he did not pay at least one visit to the tropics or sub-tropics.

Scientific work

The most striking feature of Buxton's output of scientific research, extending over forty years, is its diversity. There are papers dealing with pure taxonomy and descriptive entomology, papers on applied entomology in agriculture and medicine, on insect physiology and ecology—particularly on the border line between these two, where the study of physiology in the laboratory and of the

micro-climatic conditions in the actual environment serve to define the limitations to the existence of a given insect in the field. Interspersed with these are papers on the birds or on the people in the many lands he visited, on geography and ethnology.

During the period of his most intensive concentration on research, the twelve years before the second world war, Buxton's main interest was in those physiological factors which determine the survival and increase of insect populations under different conditions of climate, particularly the effect upon them of different temperatures and humidities. He made clear that for many purposes the saturation deficiency of the air is the most informative measure of its drying power in relation to the insect, and he repeatedly emphasized the importance of considering the 'micro-climate'—the climate in the actual spot when the insect rests—rather than the climate of the meteorologist.

In my view, however, Buxton's claims to distinction do not lie in the realm of experimental science. Without question he did useful work in that field. His experimental work on the relation of insects to climate is not negligible in itself and its influence on the work of others perhaps even more important, both in his immediate circle and among entomologists in general. His contributions to the applied entomology of filariasis, malaria and the louse-borne fevers were imaginative and timely. But these do not by themselves represent an outstanding achievement.

It is probably true to say that, except in so far as they contribute to theories and generalizations, the scientific mind is not interested in facts. But for Buxton, I believe, the scientific theory had less appeal than the facts themselves. This attitude is characteristic of the naturalist; and in my opinion it is as a naturalist that Buxton was outstanding. Whatever he might be doing, in whatever part of the world he might be, he would be sure to produce observations on the local natural history of great interest and originality. His war-time experiences in Persia and Iraq, and after the war in Palestine, resulted in the production of his book on *Animal life in deserts*. This has become a classic; and after a continuous demand lasting for thirty years, it was reprinted in 1954. In Samoa, apart from the extensive field work required for the *Insects of Samoa*, Buxton made the remarkable discovery of a truly marine Chironomid midge, *Pontomyia natans* Edwards, in which not only is the larva adapted to developing in salt water (a phenomenon not entirely without precedent) but the adult midge itself is a submarine creature which swims actively in the strong tidal currents.

In every country he visited, Buxton contributed useful observations on the birds and plants; and these catholic interests extended to the human populations. In 1921 he was writing on the way of life of the Marsh Arabs of Lower Mesopotamia; in 1926 and 1929 he writes a sympathetic account of depopulation in parts of Melanesia and Polynesia. And his more professional studies on the incidence of malaria and filariasis in the South Pacific are illuminated at every point by an understanding of the habits and outlook of the local population.

This feeling for natural history, ethnology and geography was coupled with an interest in language. Buxton early mastered Arabic and this helped greatly in putting him *en rapport* with Arab people both in the Iraq and Palestine days and

later in Egypt. And when in Samoa he went some way towards mastering that difficult language—the 'pidgin' of the South Seas.

These wide interests in anthropology, geography and animal and plant ecology provided the ideal foundation on which to base a really comprehensive account of the tsetse fly problems of tropical Africa. Before the war of 1939 Buxton had started on an ambitious text-book of medical entomology. It was never completed. The chapter on the louse was quickly turned into an admirable practical monograph and published as *The louse* soon after the outbreak of the war. The chapter on the tsetse fly grew into the great memoir of 800 pages published by the London School of Hygiene and Tropical Medicine in the last year of Buxton's life.

This *Natural history of the tsetse flies* is Buxton's *magnum opus*. Going out to Northern Nigeria for some seven months in 1933 with D. J. Lewis, he had had some first-hand experience of two species of *Glossina* under field conditions, and had made a substantial contribution to their physiology in relation to climate. This work served to emphasize that in much of its range the tsetse fly is on the border line of existence so far as climate is concerned; it is able to maintain itself only because the 'micro-climate' is within the favourable range. This discovery has provided a useful lead in the control of the tsetse fly. As T. A. M. Nash, K. M. Morris and others have found, when the undergrowth in the thickets is cleared away, without touching the larger trees, the climate in the actual spots where the tsetse fly lives and breeds becomes too unfavourable and the fly dies out. Then Buxton's extensive tours in East Africa on behalf of the Colonial Office during 1945 and 1946 gave him the opportunity of seeing the very different and diverse conditions in those regions, at first hand, and in the company of people who had been studying them for many years. Bringing his fresh outlook and wide experience to bear, and reading widely in the copious literature of the subject, he was able to achieve a masterly synthesis, in which the physiology and ecology of the many species of tsetse flies are linked with the climatic and vegetational characteristics of the various regions, and these again with the ways of life of the diverse peoples and with the incidence of sleeping sickness and trypanosomiasis in domestic animals. This book combines in a remarkable degree an immense amount of local detail with the guiding principles and generalizations derived from ecological studies. In view of Buxton's early advocacy of the study of insect physiology it is good to see the extent to which laboratory work on the tsetse fly has provided a basis for understanding its ecology.

Personal record

Patrick Buxton had a strong and distinctive personality. Completely honest, with definite personal views, he was a man to be relied upon. But for many who came into contact with him the most notable feature about Buxton was his manner —the well-known 'Buxtonian manner', a characteristic mixture of irony and display, infused with a quick wit and a well-developed sense of humour. His sharp sarcastic tongue was well known in committee. I always supposed that this manner, which could strongly antagonize those who did not see beyond it, was a

product of his school days, an intellectual method of protection against a rough world. But very many people, in all parts of the world, were not misled by this odd veneer and Buxton had friends everywhere. For the real and outstanding qualities in Buxton's character were his honesty of purpose and his thoughtfulness for others—a deep unconscious loyalty to a long family tradition.

Buxton's interests were at once broad and narrow. He was, as we have seen, intensely interested in all sides of the natural history of plants and animals, in geography, meteorology, and in the life of primitive peoples and their languages. At home he was an enthusiastic gardener, cultivating rare and unusual plants in his fine garden at Gerrards Cross and never happier than when showing them to the many friends who were invited down there or digging them up for these friends to take away. He usually wore an uncommon flower or a sprig from some rare plant in his buttonhole.

Outside his special subjects Buxton found it difficult to be interested. Music he disliked, and anyone who was interested in poetry or philosophy he would regard as distinctly abnormal; he was much attached to and well informed about the arts and crafts of Persia, less sympathetic to other forms of art. In science itself he always seemed to me to be more fascinated by the multifarious details that appeal to the naturalist than by the ideas and generalizations that are the objectives of the scientist and philosopher. In the day-to-day pursuit of his researches he usually preferred to collect facts and to sort them out with the aid of statistics than to devise experiments.

Buxton as this record has set out to show, was a great naturalist; and in common with that rare breed, he had a fine command of English and wrote in a lucid unaffected style. Even in his massive work on the tsetse fly the high quality of the writing is maintained and will ensure that this book has an honourable future. He was equally effective as a speaker, with a vivid descriptive power and a way of presenting even familiar matters in a new light.

Behind all his professional activities Buxton was a family man. Happily married at an early age, he had a family of two sons and four daughters. These have taken up very diverse careers. Only one of them, the second boy, Andrew, became a professional biologist. Andrew Buxton bore a striking resemblance to his father in general appearance and in his quick wit and lively sense of humour, so that people knew on sight whose son he was. After running the Natural History Society at Rugby and graduating at Trinity College, Cambridge, Andrew Buxton had started on research in East Africa, studying the natural history of monkeys and other potential reservoirs of the yellow fever virus—when he was suddenly struck down by poliomyelitis. The death in 1952 of this son in whom he had been able to re-live his own enthusiasms was a terrible blow to Patrick Buxton. It seemed for a time that he would never recover, and when he did so it was apparent to all his friends that many of Buxton's asperities had gone and the fine quality of his nature was there for all to see.

It is not usual to use the word tragedy when writing of the death of a man of sixty-three whose main work has been accomplished. But in his last few years Buxton was turning his attention to a new branch of natural history, that of the

Mycetophilidae—with the idea of providing a new field of interest for his retirement. He was already producing valuable results and when he died on the threshold of what promised to be a new career, those who had appreciated his kindness and unselfishness for many years felt his death as a tragedy.

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ON A NEW CYCLOPHYLLIDEAN CESTODE, *MULTICEPS SMYTHI* N.SP., FROM DOGS IN DUBLIN, EIRE

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(With 2 Figures in the Text)

The tapeworm described in this paper was collected from dogs in Dublin in April 1955 mainly for the histochemical study of a cyclophyllidean type. The results of this study are being sent for publication separately. During the course of routine identification of the tapeworm, it was found that it differed considerably from species so far described. A detailed study was, therefore, undertaken, and it proved to be an entirely new species.

MATERIAL AND TECHNIQUE

Care was taken to open the intestine in Ringer–Locke solution at 40° C. The worms were preserved in (*a*) 70% alcohol, (*b*) Bouin's fluid, (*c*) Carnoy, 10% formol, (*d*) formol calcium and (*e*) 10% formol in Ringer–Locke. Histochemical reactions on frozen and other microtome sections helped considerably to reveal the morphology of the worm. The author strongly recommends the use of histochemical methods such as the P.A.S. technique, even for ordinary routine identification. Apart from its specific advantages, it often reveals unmistakably the structural details of the tissues, so that they can be readily differentiated in sections.

DESCRIPTION

The following is a description of the new species found. All measurements are in mm. unless otherwise stated: maximum size 433 long and 4·5 broad. Scolex distinctly marked off, 0·75 long and 1·1 in maximum diameter. Suckers well developed measuring 0·29–0·4 in diameter. Rostellum 0·51 in diameter, bearing forty-four rostellar hooks disposed in two rows of alternating crowns. The large and the small hooks measure 220–238 and 140–150μ respectively. The larger hook handle is sinuous and longer than the blade. The guards are prominently projected out.

The anterior segments are short, but the succeeding ones are elongated. Genital pore alternating irregularly and located a little behind the middle of the proglottis margin. Cloaca very well developed, provided with a thick musculature and 0·11 in depth. Cirrus sac in mature segments, 0·370–0·396 × 0·15–0·167, just crossing the ventral longitudinal excretory vessel. Vas deferens greatly coiled and extended towards the centre of the proglottis. Testes 286–310 in number filling up the entire segment in between the ventral longitudinal excretory vessels; situated in front of and lateral to the ovary and even a few behind it by the side of the vitelline

glands on the aporal side only. Testes absent from the lower poral part of the segment lateral to the poral lobe of the ovary and on the poral lateral side of the vitelline glands. Maximum diameter of the testis 0·123. Vagina opening in the genital atrium very close to the opening of the cirrus sac. It is interesting to note that the vaginal canal and the cirrus canal almost touch each other, or they appear to be more or less united with each other. The vaginal course towards the longitudinal excretory canal follows a distinctive curve. A well-developed receptaculum seminis present. Ovary bilobed, the aporal lobe being usually longer. Each lobe is further composed of a large number of tubules, which are filled with numerous ova of different dimensions.

Mehlis' gland (Fig. 2, *m.gl.*) between the two lobes of the ovary towards the posterior end of the segment and measuring 0·255 × 0·158. Each gland distinguished into two zones: a peripheral zone composed of elongated cells with processes tapering towards the centre of shell gland; a central zone composed of cells in which no nuclei are seen; associated with these cells are processes of the cells of the peripheral zone. Histochemical tests on the glands show that the cells of both the zones are glandular. Vitelline gland posterior to the ovary and measuring 1·16 × 0·28. It is made up of a large number of tubules consisting of vitelline cells. A vitelline cell is almost spherical in form; the cytoplasmic contents are concentrated in a crescent-like pattern, with a nucleus lodged in them; a major part of the cell is occupied by a special type of protein. Uterus in partly mature segments, a simple, elongated chamber commencing from the shell gland and extending a little beyond the centre of the segment. On its lateral side it shows small irregular outgrowths; these are rudiments of the future lateral branches to be fully established in the gravid segment. The fully gravid uterus is voluminous and largely obliterates the genital organs. The main stem of the gravid uterus extends up to the anterior border of the segment and it gives out on each side 16–19 lateral branches, which are fairly close together. The lateral branches tend to form secondary branches, which extend to the lateral margin of the segment. Eggs mostly spherical, 0·036–0·04 in diameter. Each egg encloses an embryophore which is normally 0·010 thick. Oncosphere and embryonic hooks measure 0·018–0·010 (diameter) and 0·011–0·014 (entire length) respectively. Closely surrounding the embryophore is a thin-walled capsule bearing a bulge on one side, which contains fatty globules.

The sinuous handles of the large rostellar hooks and the reflexed loop on the vagina show that this species belongs to the genus *Multiceps* Goez, 1782. The smaller size and the smaller number of the rostellar hooks exclude this species from the following species: *M. brauni* Setti, 1897; *M. crassiceps* Zeder, 1800; *M. gaigeri* Hall, 1916; *M. packi* Christenson, 1929; and *M. serialis* (Gervais, 1847). Among these *M. gaigeri* is further characterized by the possession of a comparatively smaller number of testes. In *M. polytuberculosus* Megnin, 1880, *M. radiatus* Joyeux, Richet & Schulman, 1922 (reported from the coenurus) and in *M. spalacis* Joyeux, 1923, the exact number of the rostellar hooks is not known, but the smaller size of the large and small rostellar hooks easily distinguish these forms. *M. glomeratus* Railliet & Henry, 1915, reported by Turner & Leiper (1919), the

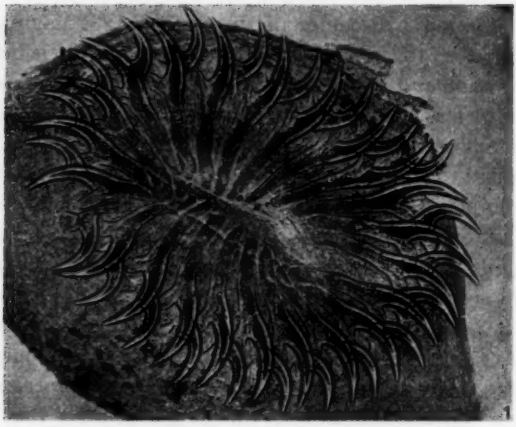

Fig. 1. Rostellar hooks (complete crowns).

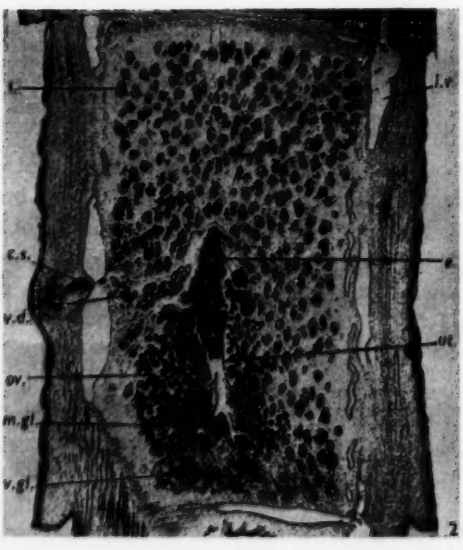

Fig. 2. Mature proglottis (facial section), P.A.S. technique.

Key to lettering on Figs. 1 *and* 2. *c.s.* cirrus sac; *e.* eggs; *l.v.* longitudinal excretory vessel; *m.gl.* Mehlis' gland; *ov.* ovary; *t.* testis; *ut.* uterus; *v.d.* vas deferens; *v.gl.* vitelline gland.

adult form of which is unknown, had rostellar hooks which were imperfectly chitinized in the immature material available, so that this species may be ruled out. *M. clavifer* Pagenstecher, 1877, *M. lemuris* Cobbold, 1861, *M. macrantha* Clapham, 1942 (which has rostellar hooks with bifid handles) and *M. otomys* Clapham, 1942, are characterized by large rostellar hooks of one size only and their size is not comparable with that of the hooks of the species here described. *M. multiceps* Leski, 1780, like the species here described, shows wide variation in the number (9–26) of its uterine branches, but it is clearly distinguished by the smaller number and size of its rostellar hooks, by the difference in their shape (length of handle, position and deflexion of the guard and the curvature of the blade), and by the smaller number and the location of the testes (the testes in the species here described are entirely absent from the extreme poral area lateral to the poral lobe of the ovary and the poral part of vitelline gland). *M. twitchelli* Schwartz, 1914, a curious branching coenurus from the lungs of the porcupine, approaches the present form in the size of its smaller rostellar hooks, but it differs in the possession of a smaller number (30–36) of rostellar hooks and in the smaller size (189–198μ) of the large rostellar hooks, together with the smaller number (7–9 pairs) of uterine branches. The polycephalic larva (*Hydatigera taeniformis* Southwell & Kirshner, 1937) which is the larval form of *M. crassiceps*, is the only form in which the number of the rostellar hooks is exactly the same as it is in the species here described, but in the former the hooks are enormously enlarged and show a great contrast.

The species here described thus differs from all other known species. It is, therefore, necessary to give it a new specific name. I have great pleasure in naming it *M. smythi* n.sp., after Prof. J. D. Smyth.

Great confusion exists in separating the two genera *Multiceps* Goez, 1782 and *Taenia* Linnaeus, 1758 because of great similarity in their morphology. The sinuous handle of the large rostellar hooks and a reflexed loop of the vagina form difficult distinguishing characters in *Multiceps*. In the diagrams of several species described no trace of a vaginal loop appears. At the same time the larger rostellar hooks of several species classified in the genus *Taenia* bear sinuous handles. These complexities and the fact that the larval form is unknown, make it difficult to devise a natural separation of the two genera.

SUMMARY

A general morphological account of *Multiceps smythi* n.sp. from the local dogs in Dublin is given. This new species is characterized by forty-four rostellar hooks arranged in two rows of alternating crowns. Testes 286–310 in a single segment, occupying all parts of the segment in between the longitudinal excretory vessels, except the poral, posterior portion of the segment. Vagina opening posterior to the cirrus sac. Receptaculum seminis present. Uterus with 16–19 lateral branches on each side. Onchosphere enclosed in an embryophore, which is itself enclosed in a thin-walled capsule which has a bulge on one side containing fatty globules. The histological structure of Mehlis' gland and of the vitelline glands is also described.

I am greatly indebted to Professor J. D. Smyth for assistance and facilities provided to carry out the work. Sincere thanks are also due to Mr D. D. Glen for preparing the microphotographs.

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A MORPHOLOGICAL AND HISTOCHEMICAL STUDY OF EGG FORMATION IN A CYCLOPHYLLIDEAN CESTODE

By L. N. JOHRI*

The work concerned in this paper was carried out in the Department of Experimental Biology, Trinity College, Dublin

(With Plate I and 1 Figure in the Text)

Considerable attention has been paid to the general anatomy of Platyhelminths with the result that the gross morphology of the different organs is well known. Our knowledge concerning the histochemistry of these organs and their role in the contribution for the final formation of the egg is much less advanced. In trematodes, turbellarians and pseudophyllidean cestodes, the evidence suggests that the egg-shell is made up of sclerotin, a quinone-tanned protein (Stephenson, 1947; Vialli, 1933–4; Nurse, 1950; Romanini, 1947; Valcurone, 1953; Smyth, 1954). No information is available as to the nature or means of formation of the 'egg' in the cyclophyllidean cestodes, and since the 'egg-shell' in this group is in reality an embryophore and not homologous with the true egg-shell (capsule) of the pseudophyllidean cestodes or trematodes, it might be predicted that its nature and method of formation would be different.

The present paper is an attempt to study the histochemistry of the organs concerned in the egg formation in *Multiceps smythi* n.sp. collected from local dogs in April 1955.

MATERIAL AND TECHNIQUE

Dogs were opened as soon as possible after death. Owing to transport and other technical difficulties, some 30 min. elapsed before autopsies were possible. While removing the parasites, care was taken to open the intestine in Ringer–Locke solution at 40° C. temp. The worms were later preserved as soon as possible in (*a*) 70% alcohol, (*b*) Bouin, (*c*) Carnoy, (*d*) 10% formol, (*e*) formol-calcium (Baker's formaldehyde calcium), and (*f*) 10% formol in Ringer–Locke. Transverse, longitudinal and facial sections of 5–7 μ thickness were prepared. Formol fixatives in general gave the most satisfactory results, although Carnoy gave fairly good structural details. Formol-preserved material was also embedded in 25% gelatine, and hardened in formol vapour for nearly 8 weeks before cutting frozen sections at 10–15 μ. Histochemical methods in general followed the procedures of Pearse (1953) with a few exceptions.

Methods for protein

The Millon, xanthoproteic and biuret methods used for proteins were those of Serra (1946). The bromo-phenol-blue method for protein and basic protein was that of Mazia, Brewer & Alfert (1953). Polyphenol oxidase was tested for by incubating whole proglottids fixed in 70% alcohol, in 0·1% catechol at 40° C. for 90 min. (Smyth, 1954).

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MORPHOLOGY AND HISTOCHEMISTRY OF GENITALIA

Ovary

Morphology. The ovary has the typical taenioid form of two distinct and unequal lobes. Each lobe is made up of numerous crowded tubules which contain a large number of ova of different dimensions. Each ovum consists of a single nucleus and granular cytoplasm.

Histochemistry. The ovary gives a positive, but not strongly positive reaction with Millon's reagent, but the result is not sufficiently sharp to enable the protein to be localized intracellularly. After P.A.S., the ova are found to contain a prominent area (*p.a.* Text-fig. 1D) which stains an intense yellow, suggesting the presence of acidophil proteins. This area is apparently easily masked, as it has not been seen with standard histological procedures. Aqueous bromophenol blue (without Hg) gives a faintly positive result, indicating only a small amount of basic proteins present.

Vitelline cells

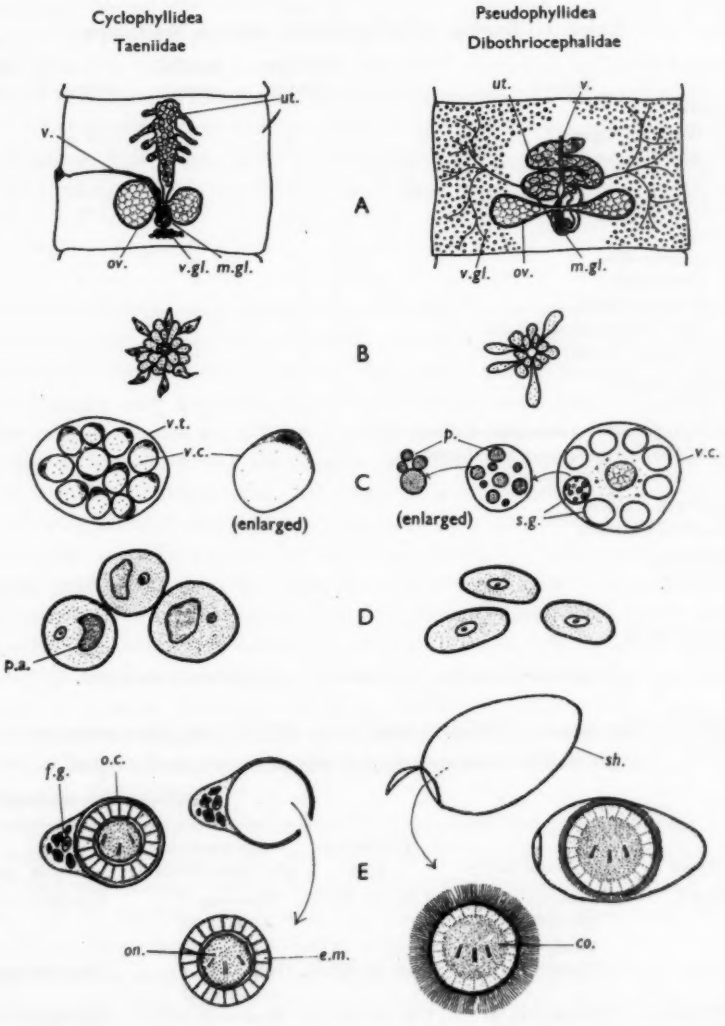
Morphology. The vitellaria have the typical taenioid position forming a single mass posterior to the ovary (Pl. I, fig. 6); unlike the pseudophyllidean forms they do not extend to the lateral fields. Histologically, they consist of large numbers of tubules together, containing numerous vitelline cells. Each cell (Text-fig. 1C) is nearly spherical in form with the nucleus and cytoplasmic contents shifted to one side in a crescent-like position, leaving a circular central area free.

Histochemistry. On theoretical grounds, on account of the absence of a true external egg-shell, it can be predicted that the vitelline cells of cyclophyllidean cestodes should be free from phenolic shell material, such as is found in the trematodes and pseudophyllidean cestodes. This speculation is borne out in practice, and the tests for phenols yield constantly negative results (Table 1). The material in the vitelline cells is likewise fat-free but gives some weakly proteic reactions (i.e. Millon). The spherical area, excluding nucleus and the granular cytoplasm, stains yellow with the full P.A.S. technique, indicating the presence of acidophil proteins. The cells give negative results for fat (Sudan Black) and polysaccharides (P.A.S.).

Mehlis' gland

Morphology. Mehlis' gland is located between the two lobes of the ovary towards the bottom and is distinguishable into two distinct regions. The peripheral region consists of enormously elongated glandular cells with prominent nuclei. Each cell is connected with a tapering process opening into the ootype located in the central region of the gland. The whole forms a complex jumbled mass and it is only possible to locate the processes by careful study of serial sections. The central mass consists of an aggregation of cell-like structures which have no nuclei. Intermingled with these structures are the tapering processes of the cells already mentioned.

Histochemistry. The cells of Mehlis' gland are unusually unreactive histochemically. They give faintly positive protein reactions with Millon and bromophenol blue, but negative results for phenols, polyphenol oxidase, disulphides and lipids.



Text-fig. 1. Comparative chart (diagrammatic) of the genitalia concerned in the egg formation in Cyclophyllidea and Pseudophyllidea. Data for the Pseudophyllidea taken from Smyth (1956). A, mature segment; B, Mehlis' gland; C, vitelline cells; D, ova; E, Egg, mature and hatching. *co.* coracidium; *e.m.* embryophore; *f.g.* fat globules; *m.gl.* Mehlis' gland; *o.c.* outer capsule; *on.* onchosphere; *ov.* ovary; *p.* protein; *p.a.* prominent area; *s.g.* shell globule; *sh.* shell; *ut.* uterus; *v.* vagina; *v.c.* vitelline cell; *v.gl.* vitelline glands; *v.t.* vitelline tubule.

With the P.A.S. technique, however, the contents of the cells are *very strongly positive* as is the glycogen in the parenchyma. After digestion with saliva or α-amylase (for details see Table 2), the P.A.S.-positive material is removed from the parenchyma but the contents of the Mehlis' gland cells are unaffected and continue to give a positive P.A.S. reaction. Little difference was found between

Table 1. *Results of histochemical tests on* Multiceps

Tests	Vitelline glands	Mehlis' gland	Ovary	Embryophore
Proteins:				
1. Millons reagent	++	+	++	+
2. Xanthoprotein	?	?	?	?
3. Biuret	?	?	?	?
4. Bromophenol blue	+	+	+	+
Phenols:				
1. Argentaffin	–	–	–	–
2. Chromaffin	–	–	?	–
3. Ferric chloride	–	–	–	–
4. Ammonium molybdate	–	–	–	–
5. Sodium iodate	–	–	?	–
Polysaccharides:				
1. P.A.S.	–	++	–	–
2. P.A.S. (after α-amylase digestion)	–	++	–	–
3. P.A.S. (after α-amylase digestion and hyaluronidase)	–	++	–	–
Polyphenol oxidase				
Catechol reaction	–	–	–	–
Disulphides:				
P.F.A.S.	–	–	–	++
Lipids:				
Sudan black	–	–	–	–

++ strongly positive; + positive; – negative; ? doubtful.

Table 2. *Reactions of Mehlis' gland to the P.A.S. test, after treatment with enzymes. The reactions of glycogen are given as a control*

			P.A.S.-positive material	
Enzyme	Concentration	Exposure time	Parenchyma (glycogen-control)	Mehlis' gland
α-Amylase*	5 mg/ml. in M/15-phosphate at pH 6	1 hr. at 37° C.	Reaction destroyed	Reaction unaltered
Saliva	Changed twice	½ hr. at 22° C.	Reaction destroyed	Reaction unaltered
Taka-diastase	25 mg./ml. in M/15-phosphate buffer at pH 6	1 hr. at 37° C.	Reaction only partly destroyed	Reaction unaltered
Hyaluronidase† (after α-amylase treatment above)	1 mg./ml. in 0·85-NaCl	3 hr. at 37° C.	Reaction already destroyed by amylase	Reaction unaltered

* Product of Light and Co., Colnbrook, Bucks, England.
† 'Hyalase', product of Benger Laboratory, Cheshire, England.

the effects of saliva or α-amylase, both effectively stripping the parenchyma of its glycogen. Crude enzyme preparations (e.g. taka-diastase), even at high concentrations, in general gave variable and unsatisfactory results. α-Amylase tended to loosen the sections from the slides, a difficulty overcome by treating slides in a horizontal position in a Petri dish. According to Pearse (1953), P.A.S.-positive materials may be divided into four groups: I, polysaccharides; II, mucopolysaccharides; III, muco- and glycoproteins; IV, glycolipids. Since the secretion of Mehlis' gland is resistant to amylase digestion, it is not in group I; nor does it give lipid reactions, a result which eliminates group IV. It can be concluded, therefore, that the secretion belongs either to group II or III, that is, acid mucopolysaccharides, hyaluronic acid, chondroitin sulphuric acid, mucoproteins, or simple basic proteins. The interpretation of these results is further treated in the discussion.

Eggs

Morphology. In the early condition, after they have reached the uterus, the ova possess a thin transparent covering. Enclosed in them are a number of micromeres containing distinct nuclei. The uterus at this stage is incompletely developed having only a few short lateral branches. At a later stage of development the uterus establishes a fully formed median chamber together with the typical lateral branches. By this time, the embryophore with a thick striated 'shell' is completely developed, enclosing the onchosphere with six embryonic hooks. Closely surrounding the embryophore is a thin-walled capsule bearing a bulge on one side. This outer capsule is not preserved by normal fixation and embedding procedures, and its presence was not suspected from the study of routine sections. The appearance in wax sections is typically shown in Pl. I, figs. 1 and 2, each ovum being surrounded by thickened embryophore which is virtually free from any visible outer covering. In frozen sections of formaline-fixed material embedded in gelatine there is a striking difference, each ovum being clearly enclosed in an outer capsule (Pl. I, fig. 5), which also encloses a conspicuous cytoplasmic bulge (clearly seen in Pl. I, figs. 5 and 6), rich in lipids which stain intensely with Sudan black.

Histochemistry. Early ova, before the embryophore is developed, stain a yellow colour with P.A.S., suggesting acidophil proteins. The embryophore gives a definite—but not strong—reaction with Millon and bromophenol blue indicating proteins. Of the structural proteins, common in invertebrates, only three are likely to form the embryophore: chitin, sclerotin, or keratin.

Negative results were obtained for chitin, using the chitosan nitrate test. If the embryophore was sclerotin, or quinone-tanned protein, it should give tests for polyphenol oxidase. Tests for phenols were completely negative, as was also the catechol incubation test of Smyth (1954). Clearly, the embryophore was not a quinone-tanned protein. Finally, keratin was tested for. This is essentially a test for sulphur. The test most readily adapted to this material was the performic acid Schiff reaction (P.F.A.S., Pearse, 1953), which is a method for disulphides. With this technique, the embryophore gave a strongly positive reaction, staining

bright red-pink, and standing out brilliantly against the almost colourless parenchyma (Pl. I, figs. 1 and 2). Whereas this test must be interpreted with caution, taken together with the negative results for sclerotin and chitin, it strongly suggests the presence of —SS— bonds, and it may be tentatively concluded that the embryophore is keratin. Owing to shortage of materials and various technical difficulties, tests for SH groups were not carried out.

DISCUSSION

From the results enumerated above, it is evident that the histochemistry of the genitalia of *Multiceps* is in great contrast with that of the pseudophyllidean groups. According to Smyth (1954, 1956) and Johri & Smyth (1955), the egg-shells and the vitelline globules in the vitelline cells of several pseudophyllidean forms give a strong positive reaction for polyphenol oxidase, phenols and proteins. Vialli (1933) has also shown that the vitellaria of *Diphyllobothrium erinacei* and *Triaenophorus nodulosus* give positive reactions for phenols. The egg-shell in these forms may thus be considered to be a quinone-tanned protein or sclerotin such as occurs in many other invertebrates wherever a tough resistant protein is required, e.g. the ootheca of *Periplaneta* (Pryor, 1940; Brunet, 1951–2); the byssus of *Mytilus* (Brown, 1952; Smyth, 1954); the cuticle of *Ascaris* (Brown, 1952). According to recent work by Pryor (1955) and Brunet & Kent (1955), the chemistry of the quinone-tanning process is much more involved than was originally suggested by Pryor (1940). In pseudophyllidean forms the mechanism of egg-shell formation is probably similar to that of *Schistocephalus*; the mature vitelline cells, laden with 'globules', pass into the lower uterus where, in the region of Mehlis' gland, the globules are released and run together to form a shell surrounding the ovum and the remnants of the vitelline cells. On exposure to air, the phenolic substance becomes oxidized to a quinone which 'tans' the protein material, forming a hard, stable egg-shell. In trematodes and turbellaria, the most recent evidence (Stephenson, 1947; Smyth, 1954; Rennison, 1953; Vialli, 1933; Nurse, 1950; Romanini, 1947; Valcurone, 1953) suggests that the egg-shell or capsule is formed in a similar way.

The genitalia of the Trematoda, Turbellaria and Pseudophyllidea are characterized by possessing extensive vitellaria which typically extend into lateral fields (Text-fig. 1). The extensive nature of the genitalia may be correlated with the large quantity of protein material required for egg-shell formation.

In Cyclophyllidean cestodes, on the other hand, no true external egg-shell or capsule is formed—*a morphological feature which may be correlated with the small size of the vitellaria and the absence of lateral extensions.* In *Multiceps* the vitelline cells are smaller than in the Pseudophyllidea and free from the phenolic-protein globules found in the latter group, although they contain acidophile protein material of some kind.

The failure of tests on the embryophore for sclerotin and chitin suggests the possibility that it might be 'keratin'. The positive P.F.A.S. reaction, indicating the presence of —SS— bonds, gives strong additional support to this view. The

term 'keratin' is, of course, not a precise one, and is only loosely used to include those proteins which contain a high proportion of cystine, and give a typical 'keratin-type' X-ray diffraction pattern (Brown, 1950). It is thus tentatively concluded that the embryophore of this form and possibly that of Taeniidae in general is formed of the 'keratin' type of protein.

From the material available for this work, it has not been possible to trace out the cytological origin or method of formation of the embryophore. It has been shown that the vitelline cells and the ova contain large bodies believed to be acidophile proteins, and it is possible that these bodies play some part in its formation. The small size of the cells, as well as the difficulty of preserving them adequately, makes the interpretation of the mechanism of embryophore formation a peculiarly difficult one.

Many views have been put forward as to the role Mehlis' gland plays in the egg-formation mechanism. Thus it has been suggested that the gland: (*a*) forms the outer lamella of the shell, (*b*) forms the complete shell, (*c*) hardens the newly formed shell, (*d*) performs a lubricatory function, (*e*) secretes fluid to increase the spermatozoan activity, (*f*) secretes electrolytes to release shell material from the vitelline cells. (For detailed references see Dawes (1946), Stephenson (1947) or Yosufzai (1953).)

The present paper does not *directly* provide any evidence as to the function of Mehlis' gland, but indirectly may be said to provide considerable circumstantial evidence. It has been shown here that Mehlis' gland is secreting a strongly positive P.A.S. material which is stable to amylase, and free from lipid. Although the range of substances which will give a positive P.A.S. reaction is probably not yet fully known, on the basis of Pearse's (1953) groupings, the secretion is likely to be a mucopolysaccharide or a muco- or glycoprotein. In this laboratory recent work by Smyth (1956) and Johri & Smyth (1955) have shown that Mehlis's gland of both the pseudophyllidean form *Schistocephalus* and the trematode *Fasciola*, likewise give identical P.A.S. reactions, which suggests that those organisms are secreting the same type of material. Considering the different nature of the cyclophyllidean embryophore and the pseudophyllidean egg it is clear that the function of Mehlis' gland is unlikely to be related directly with either the formation of the shell or embryophore. Further interpretation of the structure and function of Mehlis' gland must await the elaboration of more detailed methods to distinguish between the various P.A.S.-positive substances.

SUMMARY

1. The morphology and histochemistry of the genitalia concerned in the formation of the egg in *Multiceps smythi* n.sp. are described.

2. The vitelline cells are devoid of the phenolic protein globules, which occur in the vitelline cells of the Pseudophyllidea; this result is correlated with the absence of a true external egg-shell.

3. The hardened embryophore (often mistakenly termed the egg-shell) gives none of the reactions of sclerotin (tanned protein) or chitin, but gives a strong

P.F.A.S. reaction for disulphide bonds, suggesting that it is a 'keratin' type of protein.

4. The cells of Mehlis' gland secrete a material which is intensely P.A.S.-positive and is not digested by saliva, α-amylase or hyaluronidase. This suggests that it may be secreting a mucopolysaccharide or muco- or glycoprotein.

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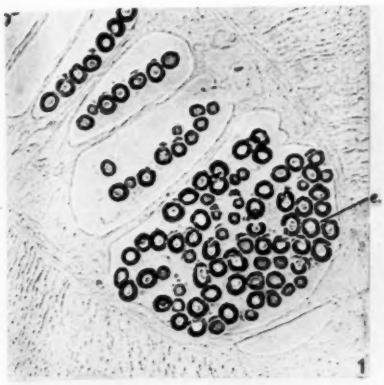
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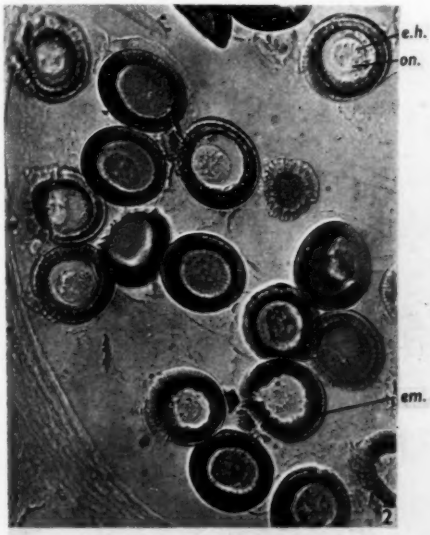
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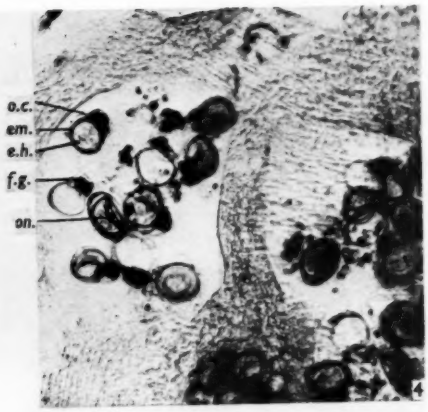
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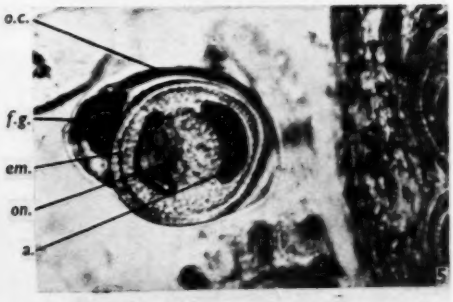
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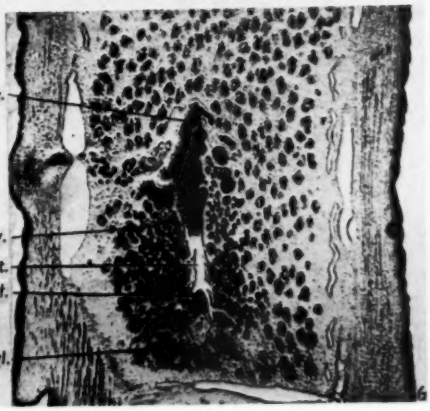
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EXPLANATION OF PLATE I

Multiceps smythi n.sp.

Fig. 1. Eggs (transverse section of gravid segment), P.F.A.S. technique.

Fig. 2. Eggs (enlarged), P.F.A.S. technique.

Fig. 3. Mehlis' gland and vitelline glands (transverse section of mature segment).

Fig. 4. Eggs (frozen section gravid segment), Sudan black reaction.

Fig. 5. Eggs (same as in fig. 4, enlarged).

Fig. 6. Transverse section of mature segment, P.A.S. technique after digestion.

Key to lettering on figs. 1–6: *a*, air bubble; *e.* eggs; *e.h.* embryonic hooks; *em.* embryophore; *f.g.* fat globules; *i.m.gl.* inner zone, Mehlis' gland; *m.gl.* Mehlis' gland; *o.c.* outer capsule; *o.m.gl.* outer zone, Mehlis' gland; *on.* onchosphere; *ov.* ovary; *ut.* uterus; *v.gl.* vitelline glands.

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THE MECHANISM OF THE ATTACHMENT OF *KUHNIA SCOMBRI* (KUHN, 1829) (TREMATODA: MONOGENEA) TO THE GILLS OF ITS HOST *SCOMBER SCOMBRUS* L., INCLUDING A NOTE ON THE TAXONOMY OF THE PARASITE

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(With Plate II and 4 Figures in the Text)

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I. INTRODUCTION

Kuhnia scombri (Kuhn, 1829) is a common parasite of the gills of the mackerel *Scomber scombrus* L. Sproston (1945*a*) gave a detailed description of the structure and mode of action of the posterior adhesive organs of this trematode, and erected for it the new genus *Kuhnia*. Later (Sproston, 1945*b*) she pointed out the difficulties of interpreting the sclerites (= skeletal supports of the posterior adhesive organs) of the Diclidophoroidea. These difficulties, which were thought to be due, among other things, to the semi-transparency of the sclerites, and to their curving in three dimensions, were held to disappear when the sclerites were regarded as modifications of the fundamental plan found in the Mazocraeidae (including *Kuhnia*). Further, Sproston thought it would be more satisfactory, as a basis for the classification of the Diclidophoroidea, to use the form of clamp skeleton rather than the number of sclerites composing it as had been advocated by Price. Accordingly she prepared a revised classification (Sproston, 1946, pp. 377–507) in which the genera and families of the group were diagnosed according to the degree of elaboration or simplification of what appeared to her to be the primitive type of clamp skeleton represented by the Mazocraeidae.

Though Sproston herself had emphasized the difficulties of interpreting the sclerites of the Diclidophoroidea (Sproston, 1945*b*, p. 191) my observations show that her own description of the sclerites of *Kuhnia*, and in consequence her description also of the adhesive mechanism, were inaccurate. Furthermore, the

erection of the new genus *Kuhnia* solely on the grounds suggested by Sproston was unwarranted. Thus, in my previous paper (Llewellyn, 1956), I accepted Dollfus's (1946) arguments for the rejection of the generic name '*Kuhnia*'. Since then Mr S. Prudhoe, of the British Museum, has very kindly drawn my attention to previously unpublished evidence that establishes the validity of Sproston's '*Kuhnia*'. For these reasons, and because of the significance that may be attributed to the structure of the posterior adhesive organs in evolutionary studies of the Diclidophoroidea, in the present paper a redescription of the adhesive mechanism is given, and evidence is submitted to validate Sproston's genus *Kuhnia*.

The descriptive terms used for the anatomy of the host gills are those employed by Goodrich (1930, pp. 490–1).

II. TAXONOMY OF *KUHNIA SCOMBRI*

Kuhnia scombri was first described by Kuhn (1829) as *Octostoma scombri*. Its subsequent history has been reviewed by Sproston (1945*a*), who proposed the new genus *Kuhnia* to include the species. This new genus was declared unnecessary by Dollfus (1946), but in an addendum to her 'synopsis of the monogenetic trematodes' Sproston (1946) reaffirmed that the replacement of *Octostoma* Kuhn, 1928 by *Kuhnia* Sproston, 1945 was indeed essential, and advanced two arguments in support of this view:

(1) That '*Octostoma* Otto, 1823' was a synonym of *Cyclocotyla* Otto, 1823, and not available for another genus.

As Dollfus (1946) has pointed out, the genus *Cyclocotyla* was first proposed in two publications in 1821 (Otto, 1821*a*, *b*) to include the species *C. bellones* Otto, 1821, and later the description was amplified and illustrated (Otto, 1923). In this last publication Otto discussed the reasons for his choice of the name 'Cyclocotyla' and for his rejection, for various reasons, of the *names* 'Octostoma', 'Polystoma' and 'Cyclostoma'. Otto could scarcely have made it clearer that he was *not* proposing 'Octostoma' for the trematode from the skin of '*Esocis bellones*', and the name therefore remained available, if required, for another genus. Thus the use of 'Octostoma' by Kuhn (1829) for *Octostoma scombri* Kuhn, 1829 was perfectly legitimate.

(2) That in any case *Octostoma* Kuhn was a synonym of *Mazocraes* Hermann, 1782, 'since their genotypes are identical'.

Here again, as Dollfus (1946) has observed, Kuhn (1829) proposed *Octostoma* for *O. alosae* Kuhn, 1829 and *O. scombri* Kuhn, 1829, without designating a type species. Thus, had no subsequent author established a type species for Kuhn's genus *Octostoma*, the proper relegation of *O. alosae* to synonymy with the type species *Mazocraes alosae* Hermann, 1782 of the genus *Mazocraes* Hermann, 1782 would have left *Octostoma scombri* Kuhn, 1829 perfectly eligible, as Dollfus contended, as the type species of *Octostoma* Kuhn, 1829 (Article 30, Rule g, of the International Code of Zoological Nomenclature). However, as Mr S. Prudhoe (personal communication) has pointed out, Stiles & Hassall (1908) designated *O. alosae* as the type species of *Octostoma*, and thus when *O. alosae* fell into synonymy with *Mazocraes alosae*, a new genus became necessary for '*Octostoma*' *scombri*.

Sproston (1945*a*) has proposed such a genus ('*Kuhnia*'), and though she made no reference to Stiles & Hassall's designation of *Octostoma alosae* as the type species of *Octostoma*, nevertheless her '*Kuhnia*' remains the proper genus for the accommodation of *K. scombri* (Kuhn, 1829) Sproston, 1945.

III. MATERIAL AND TECHNIQUES

The *K. scombri* material used in the present study was obtained from *Scomber scombrus* at Aberystwyth in August 1950 and from Plymouth in August 1954 and 1955. Specimens, some free and some still attached to the gills of the host, were fixed, some in Gilson's fluid, some in Bouin's and some in ethyl alcohol. The structure of the adhesive organs and of the associated muscles has been studied in whole mounts of free specimens, where phase-contrast microscopy was often found advantageous, and in serial paraffin sections of specimens attached to the gills. Attempts were made to dissect the sclerites of the clamps, but each clamp is only some 50–60 μ in diameter, and the results were but moderately successful. Best results were obtained with specimens that were macerated either by partial decomposition in sea water for 2–3 days, or by partial digestion with trypsin followed by staining in 0·005 % malachite green for 12–24 hr., dehydration in alcohol and mounting in Canada balsam.

Good results were also obtained by taking optical sections of material cut at 5 μ–8 μ after embedding in paraffin of melting point 63° C. Whole trematodes were stained with 0·5 % aqueous malachite green for 10 min. followed by haemalum for 5 min., with subsequent rapid dehydration in alcohols before clearing in clove oil. In this technique the sclerites (and vitelline droplets—see Smyth, 1951) retained the malachite green, while it was lost, mainly in the alcohols, from other tissues.

For sections the most useful staining techniques were found to be Heidenhain's azan (Pantin, 1946) and Masson's trichrome. The azocarmine of the former was the better for the identification of the sclerites, but the dense staining of these was achieved only at the expense of masking, to some extent, the aniline blue taken up by the tendons. During microscopic examination this masking was minimized, when required, by use of a filter consisting of a diluted sample of the azocarmine stain. In the Masson technique the much less intense staining of the sclerites with haematoxylin left them sufficiently transparent for the relatively easy identification of the tendons stained with light green.

IV. THE ATTACHMENT MECHANISM

Kuhnia scombri lies attached to the gills of *Scomber scombrus* with its body parallel to the primary lamellae and with its posterior end directed towards the gill arch, i.e. 'upstream' with respect to the gill-ventilating current of the host. Of about two dozen specimens that I have collected, all have been found attached well towards the proximal ends of the primary lamellae, i.e. in the region where, in the mackerel, the two hemibranchs are adherent to each other (Pl. II, fig. 1). In this position the parasite would be sheltered by the gill arch from the full force of the gill ventilating current. The parasite lies between the primary lamellae either

of one or of the other of the two hemibranchs of a gill, attached to the secondary lamellae either of the dorsal surface or of the ventral surface of a primary lamella (Pl. II, fig. 1). The adhesive attitude of *Kuhnia scombri* has been compared elsewhere (Llewellyn, 1956) with that of some other diclidophoroidean trematodes.

The parasite is provided with an adhesive apparatus both at the anterior and at the posterior ends of its body, but the former is probably used much more as part of the feeding mechanism than for simple adhesion to the host, and will be described elsewhere. The posterior adhesive organs are borne on a disk that is separated anteriorly by a transverse groove from the body proper, which in consequence appears to arise as a stalk from the dorsal surface of the disk. These posterior adhesive organs are of two kinds: (*a*) hooks, and (*b*) clamps.

(*a*) *The hooks*

There is a single pair of large hooks provided with well-developed muscles, and one or two pairs of smaller hooks provided with much less developed muscles. It is possible that the smaller hooks are merely survivals of the larval hooklets described by Gallien & Le Calvez (1947). Each of the large hooks (Pl. I, fig. 2, and Text-fig. 1) has a lamellate proximal portion and a distal curved tapering rod-like portion which during adhesion perforates three or four secondary gill lamellae of the host (Pl. II, fig. 3). The lamellate portion of the hook lies in a vertical longitudinal plane of the parasite (Text-fig. 1), with its long axis passing obliquely antero-ventrally and with the rod-like portion lying external to the ventral surface of the body. Near its junction with the projecting rod-like portion, the lamellate part bears an anteriorly directed spur which serves for the attachment of muscles (Pl. II, fig. 2, and Text-fig. 1).

To each hook are attached four main muscles: posterior, antero-lateral, antero-ventral and antero-dorsal. There are other well-developed muscles which, though not actually attached to the hooks, appear to be associated with them (Text-fig. 1). They comprise a broad shallow band of transverse ventral hook muscles passing transversely across the body in a ventral plane just anterior to the hooks (Pl. II, fig. 2, and Text-fig. 1), and a pair of much stouter ventro-dorsal hook muscles. The latter have their origins in the portion of the ventral body wall lying, on each side of the animal, between the posteriormost clamp and the hook of the same side. Each of these ventro-dorsal muscles then passes obliquely dorsally until, at about the level of the anteriormost pair of clamps, the fibres of the inner half of each muscle cross to the other side, the four halves of the two muscles then forming a powerful band of longitudinally running dorsal muscles.

The function of the posterior antero-ventral and antero-dorsal muscles of the hook appears to be the insertion and retention of the hook in the host tissues. The transverse ventral muscles could help to withdraw the hooks from the gill lamellae. However, there is no evidence that the parasite, once having established itself on the gill, does in fact change its region of posterior attachment. The transverse ventral muscles, together with the antero-lateral muscles, may serve merely to orientate the hooks during the initial perforation of the secondary gill lamellae, and subsequently may have a skeletal function in the general bracing of the

posterior adhesive disk. The ventro-dorsal muscles, having their origins near to the firmly anchored hooks, could bring about the lifting of the anterior end of the body that takes place when the parasite shifts the site of application of its mouth to the host tissues.

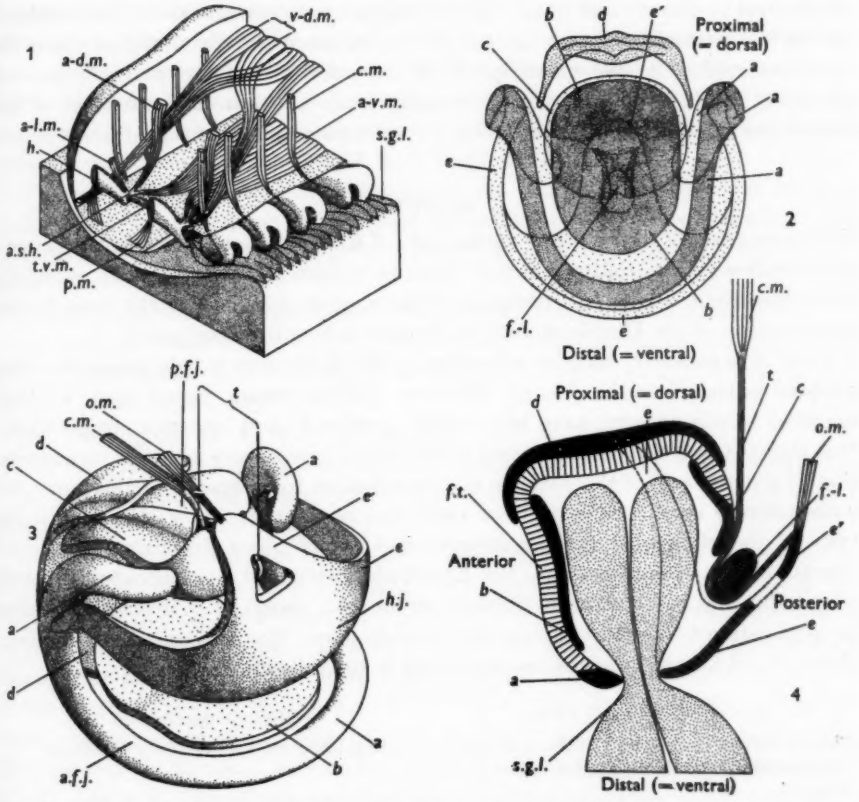
(*b*) *The clamps*

Each of the eight clamps is borne on a very short peduncle, that of the first clamp on each side being slightly longer than the rest, and each consists of a rigid arch between the arms of which is swung a hinged jaw, the hinge axis lying almost transversely to the longitudinal axis of the trematode (Pl. II, fig. 2). When the parasite is attached to its host the opening of the arch is directed ventrally and slightly posteriorly, so that one of the limbs of the arch lies anteriorly and the other posteriorly. Attachment to the host is effected by the grasping of the free distal regions of one or two secondary gill lamellae between the anterior (fixed) jaw and the hinged jaw. The posterior (fixed) limb of the arch, while contributing to the clamping mechanism, does not itself grasp the host tissues. When subjected to pressure from a cover-glass in the preparation of whole mounts, the whole clamp frequently swings backwards so that the gape becomes directed posteriorly. As a result, in ventral views of whole mount preparations (Pl. II, fig. 2), the jaws are usually seen superimposed, with the anterior lying ventral to the posterior. In this illustration the opening of the anteriormost clamp on the animal's left is directed anteriorly; all the others are directed posteriorly and present an aspect similar to that seen in Text-fig. 2. The walls of the clamp are fibrous and are supported by sclerites, the clamping mechanism being operated by an extrinsic muscle and a tendon.

The arrangement of sclerites is constant in all eight clamps (Text-fig. 3): the fixed jaws are supported by four sclerites, *a*, *b*, *c* and *d*, and the hinged jaw by a single sclerite, *e*. Sclerite *a* supports the peripheral regions of the anterior jaw, of the proximal two-thirds of the posterior (fixed) jaw, and of the arched proximal region of the clamp between these two jaws. The corresponding median regions of these same jaws are supported by three separate lamellate sclerites. Sclerite *b* supports the anterior jaw, sclerite *c* the proximal two-thirds of the posterior (fixed) jaw, and sclerite *d* the proximal region of the clamp between these two jaws. Sclerites *b* and *c* lie internally with respect to the fibrous walls of the clamp, i.e. they line the cavity of the clamp (Text-fig. 4), whereas sclerite *d* lies outside (= proximally to) the fibrous wall, i.e. between the fibrous tissue of the clamp and the parenchyma of the peduncle. Sclerites *c* and *d* have each a median longitudinal corrugation serving to increase their mechanical strength.

Sclerite *c* is specialized to form part of the clamping mechanism. For this purpose it is perforated near its distal end to allow the passage of a slender tendon from the extrinsic muscle to the hinged jaw (Pl. II, fig. 6), and furthermore its distal margin is modified to form a 'fair-lead' around which the tendon passes (Text-fig. 4). This distal end of *c* is greatly thickened so that in sagittal section it is oval (Pl. II, fig. 6). The thickened margin bears, mainly on its distal (= ventral) and anterior surfaces, a pair of ridges (flanges) that enclose a groove to accommodate the tendon (Pl. II, fig. 8).

The movable jaw is supported by the single sclerite *e* (Text-fig. 3) which passes along its lateral and distal margins, and proximally is freely hinged to the proximal regions of *a*. In its distal portion sclerite *e* is produced posteriorly, and the extension so formed (*e'*) contains a hole which has no obvious function. It is to the proximal surface of the posterior tip of *e'* that the tendon from the extrinsic muscle of the clamp, after passing round the fair-lead of *c*, is attached (Pl. II, figs. 6, 7).



Text-fig. 1. Stereogram of posterior adhesive apparatus of *Kuhnia scombri*. *a-d.m.* antero-dorsal muscle; *a-l.m.* antero-lateral muscle; *a.s.h.* anterior spur of hook; *a-v.m.* antero-ventral muscle; *c.m.* closing muscle of clamp; *h*, hook; *p.m.* posterior muscle; *s.g.l.* secondary gill lamella; *t.v.m.* transverse ventral muscle; *v-d.m.* ventro-dorsal muscle.

Text-fig. 2. Sclerites of a clamp of *Kuhnia scombri*, reconstructed from two superimposed photographs, one mainly of the anterior jaw (heavily stippled), the other mainly of the posterior jaw (lightly stippled). *a*, *b*, *c*, *d*, *e*, *e'*, sclerites as described on p. 34. *f.-l.* fair-lead.

Text-fig. 3. Stereogram of a clamp of *Kuhnia scombri*. *a*, *b*, *c*, *d*, *e*, *e'*, sclerites as described on p. 34. *a.f.j.* anterior fixed jaw; *c.m.* closing muscle of clamp; *h.j.* hinged jaw; *o.m.* opening muscle of clamp; *p.f.j.* posterior fixed jaw; *t*, tendon.

Text-fig. 4. Median vertical section of a clamp of *Kuhnia scombri* to show clamping mechanism. *a*, *b*, *c*, *d*, *e*, *e'*, sclerites as described on p. 34. (*b* is in the anterior fixed jaw, *c* in the posterior fixed jaw, and *e.e'.* in the hinged jaw.) *c.m.* closing muscle of clamp; *f.-l.* fair-lead; *f.t.* fibrous tissue; *o.m.* opening muscle of clamp; *s.g.l.* secondary gill lamella; *t*, tendon.

Each clamp is closed by the action of its own single extrinsic muscle. This muscle (Pl. II, fig. 4) passes from near the dorsal body wall of the trematode directly ventrally to the peduncle of its clamp, where it is attached to a tendon of about 2–3 μ diameter. At this region of junction several small muscles (Pl. II, fig. 4) radiate in a horizontal plane. The tendon passes distally into the clamp (Pl. II, fig. 6), lying posterior to sclerite *c* as far as the perforation in this sclerite, whereupon it becomes threaded through this hole to pass first along the anterior, then the ventral (= distal), and finally back along the posterior surface of the fair-lead. In the last part of its course the tendon lies between the distal end of *c* and the proximal surface of the extension *e'* of the sclerite *e* of the movable jaw. A relatively small muscle passing proximally from the posterior extension of the hinged jaw (Text-fig. 4) is antagonistic to the closing muscle of the adhesive organ.

V. DISCUSSION

The description of the adhesive mechanism of *Kuhnia scombri* given in the present paper differs substantially from that given by Sproston (1945*a*), and so, especially since Sproston used her interpretation of the clamps of *Kuhnia* as the basis for her classification of the Diclidophoroidea, the accounts will be compared.

First, it is necessary to draw attention to the differences in topographical terms adopted in regard to the clamps: Sproston used the terms 'dorsal' and 'ventral' for what in this account have been called 'posterior' and 'anterior' respectively. It is thought that the terms selected in this paper give a more accurate representation of the attitude of the parasite during adhesion to its host. Furthermore, the nomenclature used here may be of assistance in comparative studies, e.g. if the jaws of the clamps of *Kuhnia scombri* and *Diclidophora denticulata* be shown eventually to be homologous, the hypothesis advanced by Cerfontaine (1896) concerning a possible rotation through 90° of each clamp of *D. denticulata* before its application to the host tissues may be redundant. The two schemes of nomenclature used for the sclerites are compared in the following table:

Sproston (1945*a*, *b*, 1946)	Present description
(Clamp formed of a very thin cuticular capsule with local thickenings = various loops)	(Clamp supported by discrete sclerites)
Ventral loop	The part of *a* in the anterior jaw
Dorsal loop	The parts of *a* in the posterior (fixed) jaw
Shoulder springs	The parts of *a* between the anterior and posterior (fixed) jaws
Middle opposable piece with thin scoop-like expansion	*e* with posterior expansion *e'*
Thin cuticular capsule	Discrete sclerites *b*, *c*, *d*

There is broad agreement as to the disposition of the peripheral sclerites *a* and *e*, though Sproston's view that the 'dorsal loop' is continuous distally is not confirmed (see Text-figs. 2 and 3). This posterior distal discontinuity may have phylogenetic significance in that the present single sclerite may well have arisen from two separate sclerites by fusion in the anterior distal region.

I have not found, in transverse sections, any evidence of the clip-flange relationship between *e* (='middle opposable loop') and the anterior region of *a* ('ventral loop') that is an essential part of Sproston's interpretation of the clamping mechanism. The shape of the bars *a* and *e* shown in transverse section in Pl. II, fig. 5, is quite typical; there is no trace of any mechanism for interlocking the two sclerites.

In regard to the sclerites supporting the more median regions of the clamp there is more discrepancy. Sproston described the clamp as being formed of a very thin cuticular capsule, with the 'cross-bar' at the 'anterior' (=proximal) end that had been mentioned by previous authors being merely the optical section of this capsule. In fact there are three discrete sclerites *b*, *c* and *d*, two of them, *b* and *c*, lining the clamp cavity, and the third, *d*, lying between the fibrous wall of the clamp and the parenchyma of the peduncle (Pl. II, fig. 6, and Text-fig. 4).

It is in the interpretation of the region of the capsule near the distal end of *c* that the greatest difference lies between Sproston's and the present descriptions. In this region Sproston (1945*a*, p. 191; 1945*b*, p. 178) described the course of the tendon as

(i) being applied along the 'dorsal' (=posterior) wall of the capsule;

(ii) being attached to the middle of the 'dorsal loop' (no corresponding part in present description) as it perforated the capsule through a polygonal hole in this 'dorsal' (=posterior) wall;

(iii) crossing the cavity of the clamp;

(iv) being threaded through a triangular hole in the 'middle piece' (=*e* with *e'*);

(v) finally expanding into a broad, more or less T-shaped piece that acted as a sliding catch.

Here the most important difference in the descriptions of the course of the tendon is that Sproston did not recognize the existence of the fair-lead which has the effect of looping the tendon through 180° and so of *reversing* the direction of its pull on the hinged jaw. It is of little if any significance from a functional aspect that the tendon is not in fact threaded through the hole in the sclerite *e* of the movable jaw (Pl. II, fig. 6, and Text-fig. 4). In referring to the end of the tendon as expanding into a T-shaped piece, it appears likely that Sproston observed the flanges of the fair-lead, which are, in fact, inclined towards each other anteriorly (Pl. II, figs. 4, 8). The tendon ends by becoming attached directly to the proximal surface of the posterior tip of *e'* (Pl. I, fig. 7) and there is simply no question of a T-shaped end or of a 'sliding-catch' arrangement.

With regard to the method of functioning of the clamp Sproston believed that contraction of the extrinsic muscle, acting through the agency of the tendon, would draw the hinged 'middle loop' (=*e*) towards the 'dorsal loop' (=the posterior fixed jaw). The 'ventral loop' (=anterior fixed jaw), being thought to be clipped to a flange on the 'middle loop' (=hinged jaw *e*), was then thought to be drawn towards the 'dorsal loop'. Such movement was thought to be resisted by the 'sprung shoulders of the clamp' (=the proximal regions of *a*), the spring constituting the opening mechanism of the clamp. This hypothesis involves such unlikely premises as (*a*) a 'freely hinged' jaw being fastened by a clip to part of

the same fixed (though admittedly 'sprung') jaw to which it is hinged, and (*b*) the opposable jaw (='middle loop') so arranged that it could pass *beyond* the posterior fixed jaw (='dorsal loop'), for otherwise there could be no clamping action between the jaws.

If the description of the clamp of *Kuhnia scombri* given in this paper be accepted, then Sproston's classification of the Diclidophoroidea is open to criticism. In this classification, based mainly on clamp structure, Sproston's interpretation of the clamp of *Kuhnia* is regarded as the most generalized type. Sproston thought the 'lightly cuticularized T-shaped tendon' of *Kuhnia* to be homologous with the T-shaped *sclerite* of other species, e.g. sclerite '*a*' of *Diclidophora denticulata* described by Cerfontaine (1896) and of *Cyclocotyla chrysophryi* (van Beneden & Hesse, 1863) Price, 1943 syn. *Choricotyle chrysophryi* van Beneden & Hesse described by Llewellyn (1941). As shown above (p. 37) the tendon of *Kuhnia* does not have a T-shaped end; moreover, its staining properties (p. 32) indicate that it is unlikely to be sclerotized, and in fact it is simply a functional tendon joining a muscle to a skeletal structure. It seems most improbable that this tendon is homologous with any sclerite of any other Diclidophoroidean. There may be some affinity between one of the median sclerites *d*, *b* or *c* (Text-fig. 4), and the T-shaped sclerite of *Diclidophora*, *Cyclocotyla* and other Diclidophoroideans, but this possibility cannot be accepted without a further morphological study on the whole group.

VI. SUMMARY

The manner by which *Kuhnia scombri* (Kuhn, 1829) Sproston, 1945, attaches itself to the gills of *Scomber scombrus* L. is described.

The parasite lies between the primary gill lamellae with its posterior attachment organs 'upstream' with respect to the gill ventilating current of the host.

The adhesive mechanism consists of two sets of organs which become attached to the secondary lamellae: a pair of hooks, provided with powerful muscles, perforates three or four secondary lamellae; and each of the members of four pairs of hinged clamps, operated by its own extrinsic muscle, grasps one or two secondary lamellae.

The description of the adhesive mechanism given differs substantially from that given by Sproston. Sproston's classification of the Diclidophoroidea, being based mainly on her interpretation of the structure of the clamps of *Kuhnia*, is therefore open to criticism.

It is a pleasure to acknowledge the hospitality provided by Prof. T. A. Stephenson, F.R.S., at the Department of Zoology, Aberystwyth, and by the Director at the Marine Biological Station at Plymouth.

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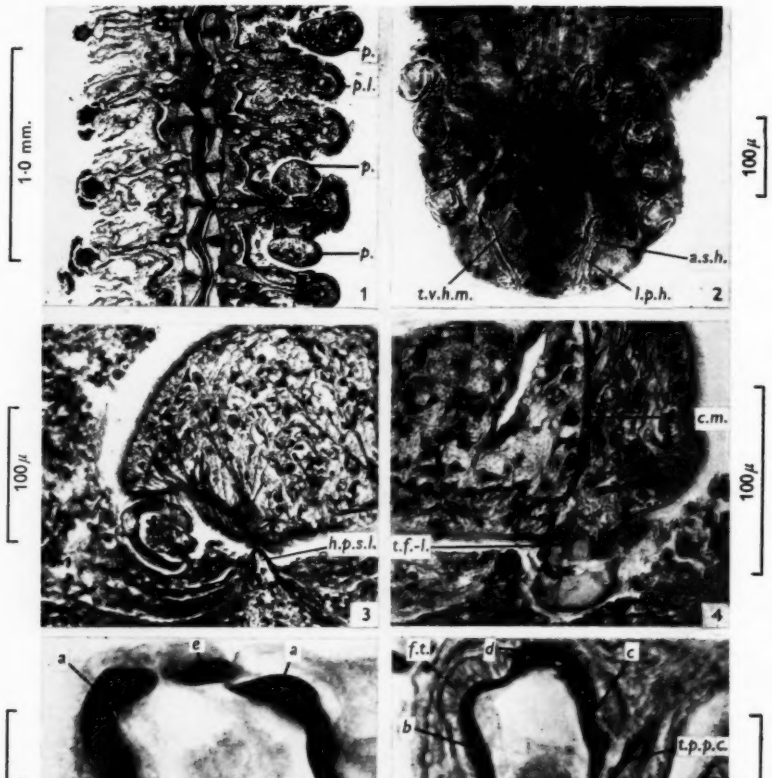
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(*Facing p.* 39)

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EXPLANATION OF PLATE II

Abbreviations: *a*, *b*, *c*, *d*, *e*, *e'*, sclerites as described on p. 34; *a.s.h.* anterior spur of hook; *c.m.* closing muscle of clamp; *f.-l.* fair-lead; *f.t.* fibrous tissue; *g.t.* gill tissue; *h.p.s.l.* hook perforating secondary lamellae; *l.p.h.* lamellate part of hook; *p*, parasite; *p.l.* primary lamella; *t*, tendon *t.a.e'.* tendon attached to *e'*; *t.c.t.* tendon cut transversely; *t.f.-l.* tendon in fair-lead; *t.p.p.c.* tendon passing through perforation in *c*; *t.v.h.m.* transverse ventral hook muscles.

Fig. 1. Transverse section of gills of *Scomber scombrus* with three specimens of *Kuhnia scombri* attached to one of the hemibranchs.

Fig. 2. Posterior adhesive apparatus of *Kuhnia scombri* in ventral view. The opening of the anteriormost clamp on the animal's left is directed anteriorly; all the others are directed posteriorly. (Specimen flattened beneath a coverglass.)

Fig. 3. Transverse section of *Kuhnia scombri* hooked and clamped to secondary gill lamellae of *Scomber scombrus*.

Fig. 4. Transverse section of *Kuhnia scombri* showing the closing muscle of a clamp, and the tendon in the fair-lead.

Fig. 5. Transverse section of clamp of *Kuhnia scombri* to show the sclerites described on p. 34.

Fig. 6. Longitudinal section of clamp of *Kuhnia scombri* to show tendon and fair-lead.

Fig. 7. Oblique section of the distal region of a clamp of *Kuhnia scombri*, passing between sclerite *e* and the fair-lead, to show the tendon attached to sclerite *e'*.

Fig. 8. Fair-lead of *Kuhnia scombri* seen distally in a whole mount preparation (× 40 phase-contrast objective used in conjunction with × 95 substage annulus).

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ON THE MORPHOLOGY OF *NEOGYRODACTYLUS INDICUS* N.G., N.SP., A VIVIPAROUS MONOGENETIC TREMATODE (FAM. GYRODACTYLIDAE) FROM *ARGULUS INDICUS* WEBER

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(With 6 Figures in the Text)

During an examination of fishes for the parasitic crustaceans, fourteen specimens of *Argulus* belonging to two different species, viz. *A. indicus* Weber, 1892 (eight specimens) and *A. siamensis* Wilson, 1926 (six specimens) were collected from the fish, *Ophiocephalus marulius* Hamilton, taken from a canal in the environs of the village Champahati, District 24 Parganas, West Bengal. Out of seven fishes so obtained only three were found to be infected with the copepod parasites. While examining these copepods, the attention of the writer was drawn to a viviparous monogenetic trematode infecting only one species of *Argulus*, viz. *A. indicus*. It was rather unfortunate that the piscine host could not be examined at that stage for the helminthic infection. About one hundred specimens of this trematode were collected from the ventral surfaces of the copepods.

A detailed study of this viviparous trematode showed that it belongs to the family Gyrodactylidae Cobbold, 1864, but not to any of the three genera of the viviparous species that are known, namely, the genera *Gyrodactylus* Nordmann, 1832; *Isancistrum* de Beauchamp, 1912, and *Gyrodactyloides* Bychowsky, 1947. A new genus has therefore been created for it and the name proposed for the new form is *Neogyrodactylus indicus* n.g., n.sp. It is interesting to note that, like the members of the family Udonellidae Taschenberg, 1879, this form is parasitic upon *Argulus* but there remains the possibility that it may also parasitize fishes.

Neogyrodactylus indicus n.g., n.sp.

The body (Fig. 1) is small, covered with a fine cuticle and almost cylindrical with slightly attenuated extremities. It may become spindle-shaped when a fully developed embryo is present. There are two small cephalic lobes (Fig. 2) containing the terminal parts of the ducts of cephalic glands at the anterior extremity of the body. The posterior extremity bears a broadly expanded disk, the opisthaptor.* The body measures 0·50–0·75 mm. in length, exclusive of the opisthaptor, and 0·12–0·21 mm. in breadth at its middle region. The opisthaptor (Fig. 3) is an almost circular disk 0·09–0·11 mm. in diameter. It has a deep antero-median cleft and a central concavity, and it bears a pair of large hooks in the central concavity and a series of 16 small hooklets arranged in a single row

* This term was first used by Dawes (1946) to denote the posterior adhesive apparatus in the Monogenea.

near the periphery. Each hook (Fig. 4*a*) consists of a stout, slightly curved shaft, a point, and two roots, the outer root being a short stump. The shaft measures 0·035–0·042 mm. from the height of the curve to the tip of the inner root, while the point is 0·012–0·017 mm. long, not following the curve. The tip of the point usually extends to a level slightly more than the anterior third of the hook. A single horizontal bar of hour-glass shape bridges the gap between the two hooks near the middle region (Fig. 3); it consists of two segments which are

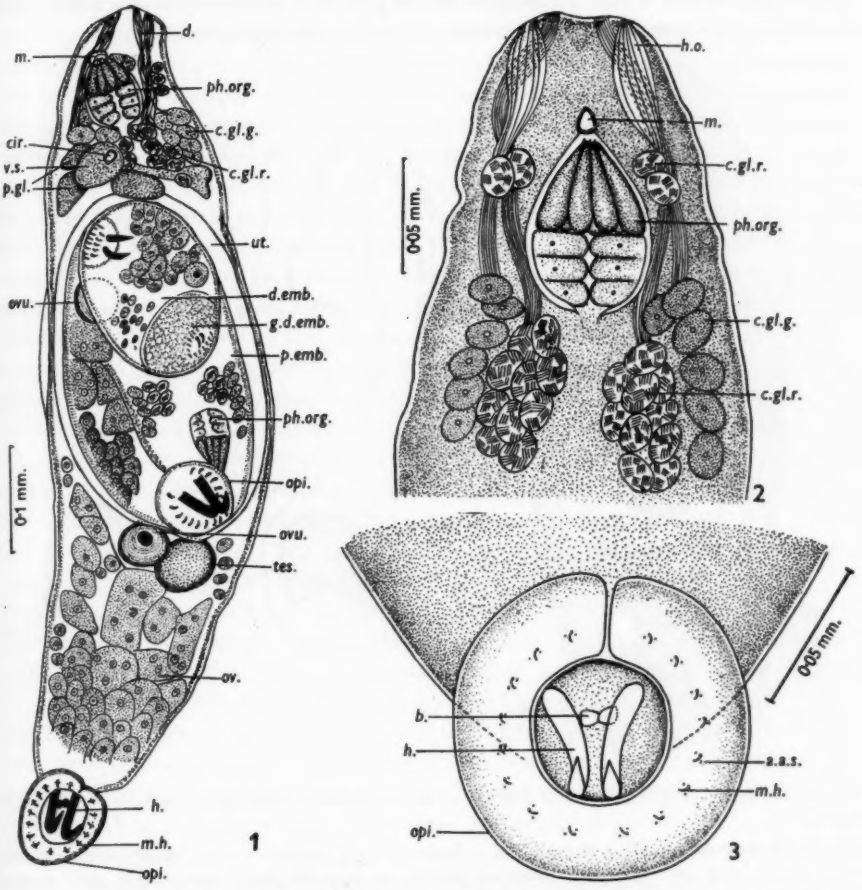

Fig. 1. Ventral view. A gravid individual. (Specimen slightly twisted.) *cir.*, cirrus; *c.gl.r.*, cephalic glands with rodlets; *c.gl.g.*, cephalic glands with granules; *d.*, ducts of cephalic glands; *d.emb.*, daughter embryo; *g.d.emb.*, grand-daughter embryo; *h.*, hooks; *m.*, mouth; *m.h.*, marginal hooklet; *opi.*, opisthaptor; *ov.*, ovary; *ovu.*, ovum; *p.emb.*, parent embryo; *p.gl.*, prostate glands; *ph.org.*, pharyngeal organ; *tes.*, testis; *ut.*, uterus; *v.s.*, vesicula seminalis.

Fig. 2. Anterior region showing the cephalic glands and head organs. *c.gl.r.*, cephalic glands with rodlets; *c.gl.g.*, cephalic glands with granules; *h.o.*, head organs; *m.*, mouth; *ph.org.*, pharyngeal organ.

Fig. 3. Posterior extremity with the opisthaptor showing the adhesive organs. *b.*, supporting bar; *a.a.s.*, accessory adhesive structure; *h.*, hook; *m.h.*, marginal hooklet; *opi.*, opisthaptor.

firmly held together and are of almost equal size, and about 0·012–0·014 mm. long. Each marginal hooklet is associated with a curved V-shaped structure (Fig. 4*b*), between the two limbs of which the hooklet is placed. This accessory structure is deeply implanted in the opisthaptor. Only the claw-like curved tips of its limbs are visible on the opisthaptor, one on either side of each marginal hooklet. It measures about 0·018 mm. in length, not following the curve. The marginal hooklets (Fig. 4*c*) are sickle-like structures consisting of two parts, a long handle which is bluntly pointed at its proximal extremity and a blade. The handle is 0·016 mm. long and is deeply rooted in the opisthaptor.

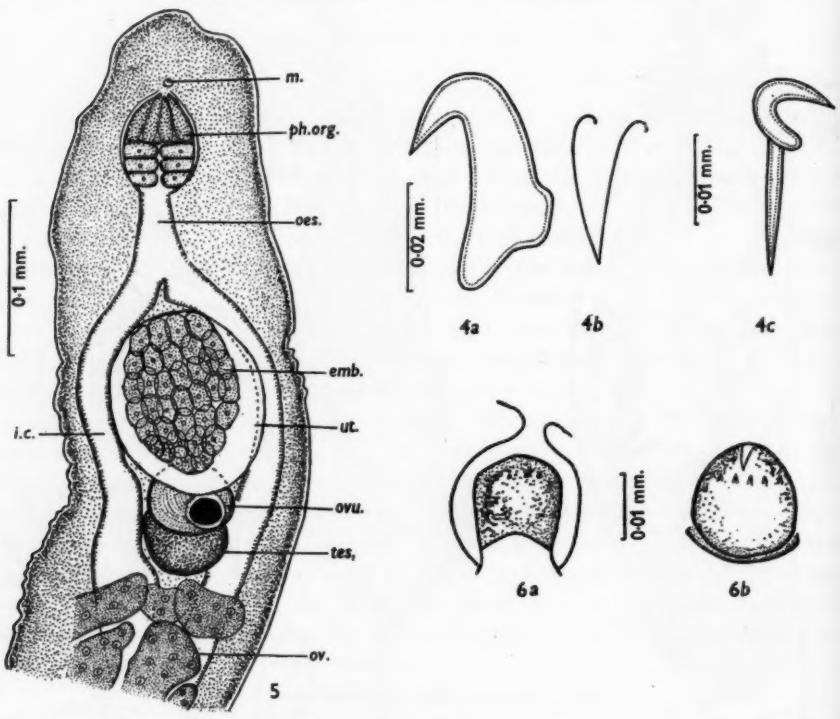

Fig. 4. *a*, a hook showing the roots (lateral view); *b*, accessory adhesive structure; *c*, marginal hooklet.

Fig. 5. An individual with a developing embryo showing the alimentary canal (ventral view). *emb.*, developing embryo; *i.c.*, intestinal caeca; *m*, mouth; *oes.*, oesophagus; *ov.*, ovary; *ovu.*, ovum; *ph.org.*, pharyngeal organ; *tes.*, testis; *ut.*, uterus.

Fig. 6. *a*, cirrus enclosed within the cirrus-sac; *b*, cirrus everted.

In the anterior region of the body there are three groups of unicellular cephalic glands (Fig. 2) on each side. These glands are of two types—one has granules while the other has rodlets—and their ducts open to the exterior at the cephalic lobes. Kikuchi (1929) described two types of similar glands in *Gyrodactylus japoncus* Kikuchi, 1929. In *Neogyrodactylus indicus* two groups of these glands are postero-lateral while the third group is antero-lateral to the pharyngeal organ.

Of the former, one group consists usually of six granular cells on each side in the lateral region of the body. The other group consists of about a dozen cells with rodlets on each side towards the median region of the body. The third group of cephalic glands is very small, consisting usually of two or three cells on each side and these also contain rodlets. Head-organs are present and they are associated with the ducts of the granular cells.

The mouth is ventral and median, and located slightly behind the cephalic lobes. It leads into a small pyriform sac having a thin wall which lodges a pear-shaped, protrusible pharyngeal organ 0·059–0·075 mm. long and 0·046–0·057 mm. broad in its basal part. The pharyngeal organ consists of anterior and posterior parts which are sharply marked off from each other by a constriction near the middle region. The anterior part comprises eight elongated conical appendages which can be moved against one another and can also be protruded through the mouth aperture like tentacles. The posterior part is of a flattened spheroidal shape and it consists of three tiers of cells placed one behind the other. The pharyngeal organ communicates with a bifurcate intestine through a short oesophagus (Fig. 5) 0·039–0·052 mm. long. The intestinal caeca run backwards along the sides of the body embracing the uterus, ovum and testis, and seem to be confluent posteriorly in the lobulated ovary. When a fully developed embryo is contained within the uterus, the intestinal caeca are pushed aside and are difficult to observe; otherwise they are easily visible.

The testis is a large globular or subglobular structure, 0·052–0·070 mm. in diameter, situated in the intercaecal field behind the uterus and it may be partly overlapped by the large ovum. In specimens bearing fully developed embryos, the testis is usually distorted by the pressure exerted by the distended uterus and it may appear oval. The accessory organs of the male genital system comprise a vesicula seminalis, a cirrus-sac and two pairs of prostate glands; they are situated in the median line in front of the uterus and ventral to the oesophagus. The vas deferens enters a large vesicula seminalis, which is full of sperms and 0·026–0·039 mm. in diameter. The cirrusa is a small, muscular, subglobular or ovoid structure, 0·018–0·020 mm. in diameter, enclosed in a spherical cirrus-sac (Fig. 6*a*). The cirrus-sac opens to the exterior through the median genital pore shortly behind the pharyngeal organ. The cirrus (Fig. 6*b*) bears a row of six to eight spinelets near its terminal part; they measure about 0·003 mm. in length. In addition to these spinelets, there is a large, rather curved 'spine' situated at the tip of the cirrus. It is much larger than the spinelets and measures 0·006–0·010 mm. in length. Usually it lies with its pointed tip turned downwards, but in some specimens it is seen in an upright condition. Possibly it is an erectile structure. The prostate glands are large organs situated at the sides of the vesicula seminalis.

The ovary* is a large, highly lobulated organ occupying almost the entire

* Yamaguti (1940) interpreted the caudally situated, lobulated structure as the true vitellaria, the saccular organ situated near the testis as the ovary; the latter, according to him, eventually functions as a pseudo-ootype. The present writer has, however, followed the usual interpretation of other workers in regarding the caudally situated, lobulated structure as the ovary and the saccular organ as the ootype.

caudal region of the body. The lobes may appear as distinct segments. The ootype is a large, saccular structure situated posterior to the uterus near the testis. It contains a single ovum which, when fully developed, measures about 0·035–0·040 × 0·049–0·052 mm. The uterus is a very large chamber occupying the middle region of the body between the intestinal caeca. Its size depends entirely on the size of the developing embryo contained within it. When the embryo is fully developed, the uterus is greatly distended and assumes a longitudinally oval shape measuring 0·365–0·415 × 0·199–0·249 mm. In such an advanced stage of pregnancy, the middle region of the body is much distended and the embryo lies in a flexed condition within the uterine cavity with its cephalic and caudal extremities side by side. Almost all the organs of the embryo are well developed, the hooks and hooklets being conspicuous. The embryo at this stage within the body of the parent contains in its uterus a second generation embryo (daughter embryo), the hooks and hooklets of which are well developed. In some specimens, the rudiments of a third generation embryo (grand-daughter embryo) are also recognizable (Fig. 1) in the uterus of the daughter embryo while the latter is still contained within the parent embryo.

DISCUSSION

The present form greatly resembles the genus *Gyrodactylus* Nordmann, 1832, in its morphology and in being viviparous. But whereas in *Gyrodactylus* there are two bars supporting the hooks, there is only one in the present form. Moreover, *Gyrodactylus* has no accessory structures associated with the marginal hooklets. The new genus *Neogyrodactylus* is therefore being proposed for the present form.

The family Gyrodactylidae Cobbold, 1864 comprises two subfamilies, viz. Gyrodactylinae Monticelli, 1892: emend. Johnston and Tiegs, 1922, and Isancistrinae Fuhrmann, 1928; the former includes the genera *Gyrodactylus* Nordmann, 1832, and *Gyrodactyloides* Bychowsky, 1947, while the latter includes only the type genus *Isancistrum* de Beauchamp, 1912. The new genus *Neogyrodactylus* is assigned to the subfamily Gyrodactylinae chiefly because the armature of its opisthaptor includes, in addition to the marginal hooklets and their accessories, a pair of hooks supported by a single bar. Hooks are not present in the subfamily Isancistrinae.

Generic diagnosis of Neogyrodactylus *n.g.*

Gyrodactylidae: Gyrodactylinae. Small, viviparous forms. Body almost cylindrical with attenuated extremities. Anterior extremity terminates in a pair of cephalic lobes. Groups of unicellular glands and head organs present in the cephalic region of the body. Opisthaptor with an antero-median cleft and sixteen marginal hooklets and a pair of hooks in the middle supported by a single bar. Marginal hooklets associated with accessory adhesive structures. Mouth anterior, median and ventral in position. Pharyngeal organ a protrusible structure consisting of two well-defined parts, an anterior part comprising eight conical appendages borne on the posterior spheroidal part. Oesophagus short. Intestinal caeca confluent posteriorly in the posterior region of the body. Ovary large and much

lobulated, occupying almost the whole caudal region of the body. Uterus a large chamber containing a developing embryo, and confined to the middle region of the body in front of the testis between the intestinal caeca. Embryo, when fully developed, usually containing a daughter embryo and, sometimes, also a granddaughter embryo. Testis single and post-uterine in position. Prostate glands, vesicular seminalis and a cirrus-sac enclosing an armed cirrus present in front of the uterus. Cirrus a small subglobular structure. Genital pore median, located behind the pharyngeal organ. Parasites of crustacea.

Genotype *Neogyrodactylus indicus* n.sp.

Key to the genera of the subfamily Gyrodactylinae Monticelli, 1892

I. Hooks supported by two bars — **Gyrodactylus** Nordmann, 1832
Hooks supported by a single bar — II

II. Complementary armature with plates and filaments absent in the Opisthaptor — **Neogyrodactylus** n.g.
Complementary armature with plates and filaments present in the Opisthaptor — **Gyrodactyloides** Bychowsky, 1947

I wish to express my grateful appreciation to Professor M. B. Lal of the University of Lucknow for his kind interest in the work, and to Dr Ben Dawes of King's College, University of London, for kindly going through the manuscript of this paper and giving valuable suggestions.

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REVISION OF THE GENUS *HYALOMMA*

II. THE SUBGENUS *HYALOMMINA**

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(With 11 Figures in the Text)

In 1901 Neumann described a new species of *Hyalomma* which he called *H. rhipicephaloides*. He based his description on two males found by Ehrenberg in Egypt in 1838 and deposited in the Berlin Museum. The host is unknown. These males are, according to Neumann, intermediate between *Hyalomma* and *Rhipicephalus*; they have the general appearance and features of the legs of *Rhipicephalus* and the capitulum of *Hyalomma*, which is, nevertheless, short. In addition, these males have a pale pigmentation ('Couleur jaune terreux' as Neumann says).

In 1919 Schulze created a new subgenus for this species: *Hyalommina*. The characters of this subgenus are, according to Schulze, the tarsi humped before their extremities, and the absence of subanal plates. In addition to the two males found by Ehrenberg and described by Neumann, Schulze found in the Zoological Museum of Berlin two males and two females collected by Dr Bruehl on 18–22 January 1912 in Wadi Ghuver from a cyst under the skin of a ibex. These specimens resemble Ehrenberg's material described by Neumann also in their pale pigmentation which Schulze thought to be characteristic of the species.

In 1928 Sharif described two new species and one new subspecies from India, *Hyalommina kumari*, *H. hussaini* and *H. hussaini* var. *brevipunctata*.

In 1936 Schulze described another new species from Kenya: *H. lewisi*. According to Schulze, subanal plates may be present or absent in this species, and in view of this alleged character it constitutes a link between the *Hyalommina* and the *Hyalomma sensu stricto*. The only species of this genus which has ever been confirmed by any author other than its discoverer is *H. hussaini*; this species has been found by Dias (1954) in Portuguese India. On the other hand, Delpy (1946, 1949) had denied the existence of the subgenus and its species. According to Delpy, all the Hyalomminas are synonyms of *Hyalomma excavatum* K. In view of the above controversy it seemed advisable to re-examine existing material together with additional specimens at our disposal.

The following material was at our disposal for the study of the *Hyalommina* problem:

H. rhipicephaloides. Two males and two females from the Zoological Museum of Berlin (kindly lent to us by Prof. A. Kaestner, the Director of the Museum), found in 1912 by Dr Bruehl in 'Wadi Ghuver' (Ein el Ghuweir?) on the western shore

* The first part of the 'Revision of the genus *Hyalomma*: Description of Koch's types' was published in the *Bull. Res. Coun. Israel*, **4**(2); 150–70, 1954.

of the Dead Sea. One of the two males found by Ehrenberg* in Egypt in 1938, which were Neumann's types for the species, was examined by us a few years ago in the Berlin Museum. One male with its nymphal exuvia and one nymph with its larval exuvia found on 16 March 1953 by M. Costa on *Acomys cahirinus* at Ein Gedi (also on the western shore of the Dead Sea), about 18 km. south of Ein el Ghuweir. One female found by Prof. O. Theodor under a stone in Wadi Ghar (also Dead Sea area) on 23 April 1946.

H. hussaini and *H. kumari*. About sixty-five specimens from India (kindly lent us by Dr G. M. Kohls of the Rocky Mountain Laboratory, and Dr G. O. Evans from the British Museum, Natural History), some of which were determined by Sharif.

H. lewisi. Males and females from Kenya determined by Schulze, now in the collection of the Rocky Mountain Laboratory.

Hyalommina rhipicephaloides Neumann, 1911

Male. The accompanying description of the male is based on the recently found specimen.

The tick is normally pigmented. The colour of the scutum is brown, as in other species of *Hyalomma*. The size of the scutum is *c.* 2·5 × 1·6 mm. It is smooth and glossy with few large dots. Cervical grooves short, but extend superficially beyond the level of the eyes. There is only a hint of the lateral grooves. Median and paramedian grooves are present and there are eleven well-separated festoons (Fig. 1 A).

The palps are relatively short (*c.* 0·4 mm.), the length of the capitulum 0·6 mm. The basis capituli is wide (0·5 mm.) with blunt lateral angles and small cornua (Fig. 1 B). The dentition of the hypostome is 3/3 with 6–7 teeth per file. The anal and adanal plates are rounded distally. There are no subanal plates (Fig. 1 D). The stigma is small with a wide tail (Fig. 1 C). The legs are pale brown. The first coxa is deeply divided, and the external process is markedly longer than the median one. The II, III and IV coxae have well-formed spurs, slightly decreasing in size from coxa II to IV (Fig. 1 F). The tarsi are humped before their distal end (Fig. 1 E).

Comparison of the newly found tick with Dr Bruehl's males shows that all three ticks are very much alike in their general appearance and most characters. The main difference is in the colour. Whereas our specimen is normally pigmented and is brown, Dr Bruehl's specimens have a pale yellow colour with only a ring of red-brown colour around the eyes. The pale colour of Dr Bruehl's specimen is not due to yellow pigment, but to very poor pigmentation of the chitin, which causes the scutum to be transparent. The stigma differs slightly in shape, the tail of the stigma of Bruehl's specimens being narrower than that of the specimen from Ein Gedi. The cervical grooves are very short and do not extend posteriorly.

Female. The two females found by Bruehl and deposited in the Zoological Museum, Berlin, are the first females found of this species. One of the females is gorged, and the other partly gorged. The partly gorged female is proposed as a lectotype and is described below.

* According to Dr W. Crome, of the Zoological Museum in Berlin, there is only one male remaining from Ehrenberg's material.

The colour of the female as a whole is pale yellow-brown like that of the males found together with it. There is a ring of red-brown pigment only around the eyes. The length of the scutum is about the same as its width. The palps are long as in other species of *Hyalomma* (*c.* 0·8 mm.), and the length of the capitulum is 1·1 mm. The basis capituli is wide (*c.* 1 mm.) and has prominent lateral projecting angles and small cornua. The dentition of the hypostome is 3/3 with eleven teeth per file

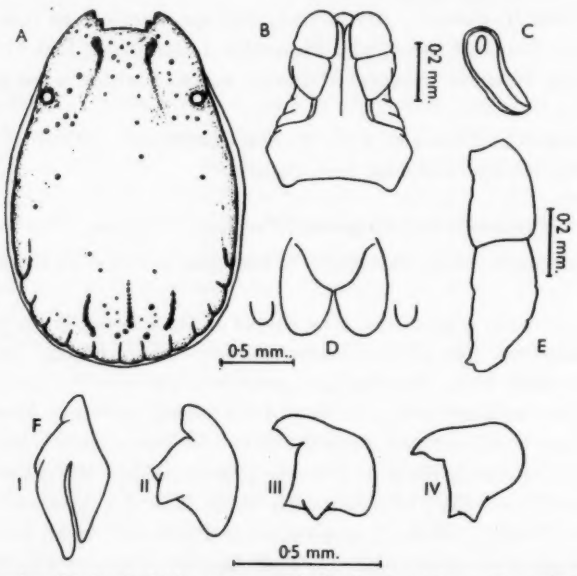

Fig. 1. *Hyalommina rhipicephaloides*, ♂, from Ein Gedi. A, scutum; B, capitulum; C, stigma; D, anal armature; E, tarsus IV; F, coxae I, II, III and IV. (The hairs on the coxae are not drawn.)

(Fig. 2A, B). The first coxa is deeply divided; the external process is slightly longer than the median. Coxae II, III and IV have a well-formed spur each, decreasing in size from coxa II to IV (Fig. 2D). The tarsi are humped (Fig. 2C). The spiracle is of the type usually found in *Hyalomma*. The mounted genital aperture has the form of a cup; the entrance into the genital tube is bare, i.e. not covered by an operculum. The upper edge of the vagina ('cup') is rounded and deeply excavated and the flaps are pointed at their anterior end (Fig. 3).

The female from Wadi Ghar does not differ in any aspect from the lectotype described.

Nymph. The nymph is described here for the first time, the description being based on the exuvia of the male found at Ein Gedi.

The nymph resembles nymphs of *Hyalomma* more than those of *Rhipicephalus*. The palps are long, the second article is about twice as long as the third. The basis capituli has a subtriangular shape. The dentition of the hypostome is 2/2, with 7–8 teeth per file (Fig. 4A, B). The length of the scutum equals approximately its width, and the eyes are behind the middle of the scutum (Fig. 4C). The first coxa

has a stout spur (Fig. 4 D). The tarsi are slightly humped. The spiracle is egg-formed, with large goblets (Fig. 4 F).

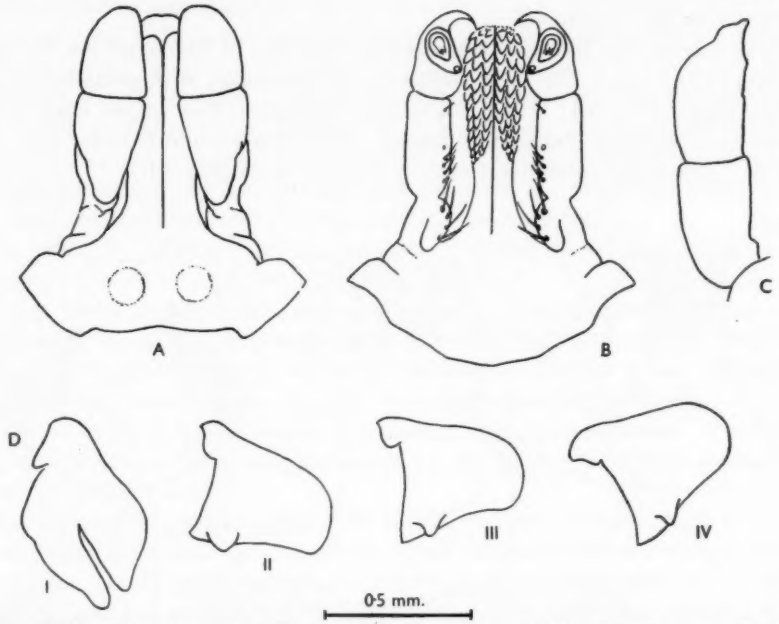

Fig. 2. *Hyalommina rhipicephaloides* ♀, lectotype from 'Wadi Ghuver'. A, capitulum dorsum; B, capitulum venter; C, tarsus IV; D, coxae I, II, III and IV. (The hairs on the coxae are not drawn.)

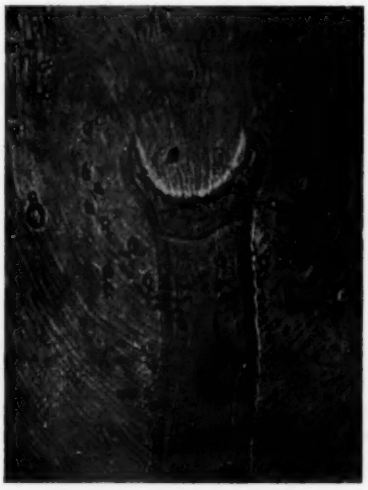

Fig. 3. *Hyalommina rhipicephaloides*, ♀, lectotype. Mounted genital aperture. *c.* × 140.

Larva. As mentioned above, together with the nymph (from Ein Gedi) which moulted to a male, there was a larva which moulted to a nymph. This nymph is also *H. rhipicephaloides*. The description of the larva will be made on the basis of the exuvia of this specimen.

The larva resembles that of *Hyalomma* more than that of *Rhipicephalus*. The palps are long (*c.* 0·1 mm.). The basis capituli is subtriangular, with acute lateral protruding angles (Fig. 5A). These angles are more acute than in the other known species of *Hyalomma* (Feldman-Muhsam, 1948). The scutum is wider than long (Fig. 5C). There is a short blunt spur on the first coxa (Fig. 5B).

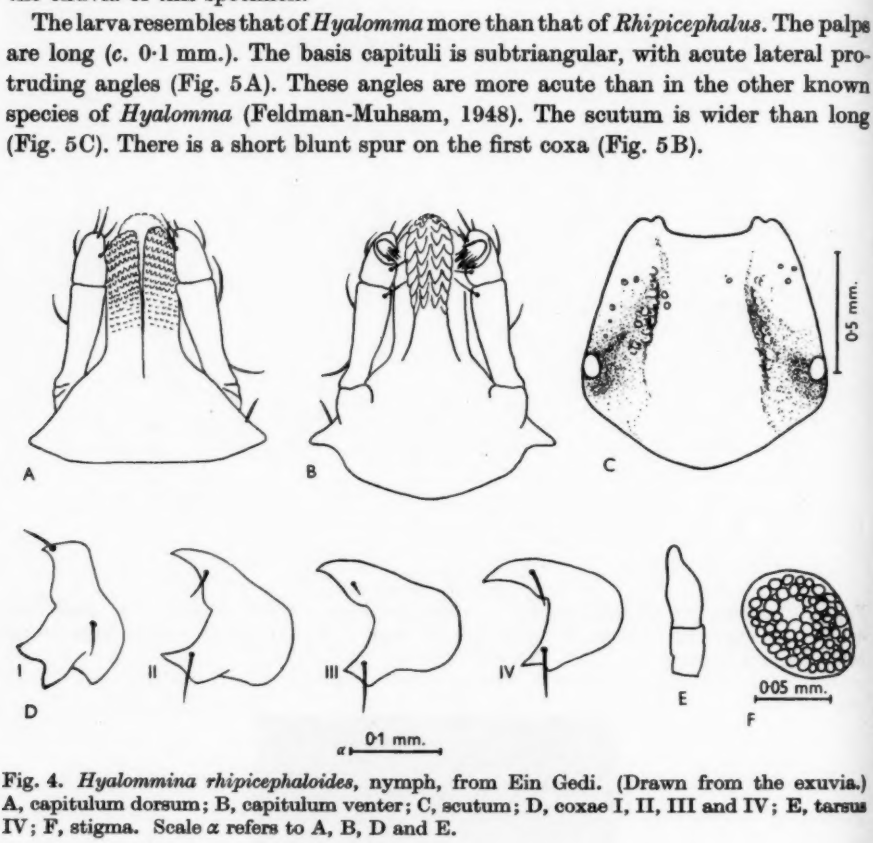

Fig. 4. *Hyalommina rhipicephaloides*, nymph, from Ein Gedi. (Drawn from the exuvia.) A, capitulum dorsum; B, capitulum venter; C, scutum; D, coxae I, II, III and IV; E, tarsus IV; F, stigma. Scale α refers to A, B, D and E.

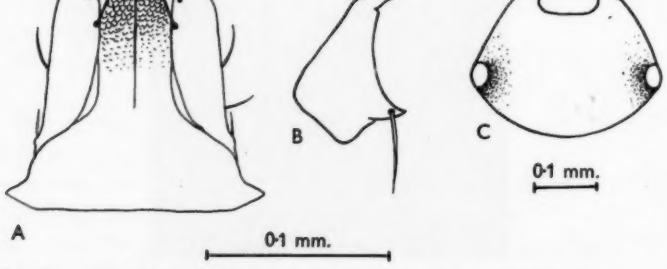

Fig. 5. *Hyalommina rhipicephaloides*, larva from Ein Gedi. (Drawn from the exuvia.) A, capitulum; B, coxa I; C, scutum.

Discussion

The type specimen described by Neumann and found by Ehrenberg has a pale colour similar to that of Bruehl's specimens. Schulze (1921) thought at first that the pale colour of the type specimen was due to the long stay of the specimens in spirit, but when he later noticed that Bruehl's specimens which had been found under the skin of the host had a similar colour, he considered the colour to be characteristic of the species. The lack of pigmentation of most of the specimens known to-day does not seem to be abnormal or due to an extraneous factor, e.g. having been kept in spirit or having remained under the skin of the host, because the unfed female found under a stone had the same colour.

The newly found male with its normal pigmentation proves that there is a wide range of variation in pigmentation in this species.

Delpy (1946, p. 80) confuses the small, abnormal specimens of *Hyalomma*, the 'pusillum' form, with *H. rhipicephaloides*. When writing about the form 'pusillum' he says: 'Lorsque la variation est très prononcée, les spécimens sont atrophiés... le scutum très voussé parfois, ne présente que des ébauches de sillons...les tarses renflés. Chez les mâles les écussons subanaux sont imperceptibles. L'aspect est celui que decrivent Neumann pour son espèce *H. rhipicephaloides*, et Schulze pour le sous genre *Hyalommina*.'

The examination of specimens of *H. rhipicephaloides* shows without any doubt the validity of this species. All the examined specimens are well formed and are not similar to any 'pusillum' form found frequently in collections of *Hyalomma*. The fact that the subanal plates are absent in the 'pusillum' forms does not invalidate this feature for distinguishing another species, i.e. *H. rhipicephaloides* in this case. (In Schulze's type of '*H. pusillum*' the subanal plates are present.)

In the 'pusillum' form all the grooves are indistinct; in *H. rhipicephaloides* the lateral grooves are almost absent, but the cervical grooves, the median and paramedian grooves are well formed. It should be noted that *H. rhipicephaloides* is not the only species of *Hyalomma* where the lateral grooves are absent. In *Hyalomma aegyptium* the grooves are even more reduced than in *H. rhipicephaloides*, and of all the grooves, the cervical are the only ones present, and even these are very short.

H. rhipicephaloides can therefore easily be distinguished from the 'pusillum' form and any other species of *Hyalomma* by the humped tarsi, the lateral protruding angles of the basis capituli (more in the females and less in the males) and the form of the anal plates of the male. The present description of the nymph and the larva adds to the distinctness and validity of this rare and interesting species.

THE INDIAN SPECIES

The Indian species of *Hyalommina* are good representatives of this subgenus and have nothing in common with the 'pusillum' form. Unlike *H. rhipicephaloides*, which is a rare species, of which only eight specimens have been identified till now, the Indian species seem to be common in India. In view of the controversy about the validity of these species and the incomplete descriptions available, it seemed desirable to study, discuss and re-describe these species.

Hyalommina hussaini Sharif, 1928

Male. All specimens are normally pigmented. The colour of the scutum capitulum and legs is red-brown. The scutum is smooth and glossy with a few large punctations, especially in the caudal and scapular areas (Fig. 6A); its shape is variable (*c.* 2·8 × 1·7 mm. or 2·5 × 1·7 mm.). The cervical grooves are short and wide; the lateral grooves are well formed, deep and long, almost reaching the eyes; posteriorly they include the fifth festoon, sometimes the fourth, too. Median groove is deep and long. Paramedian grooves are deep, wide and shorter than the median groove. The caudal area is not depressed. The anterior paramedian grooves form two wide and shallow depressions about the middle of the length of the

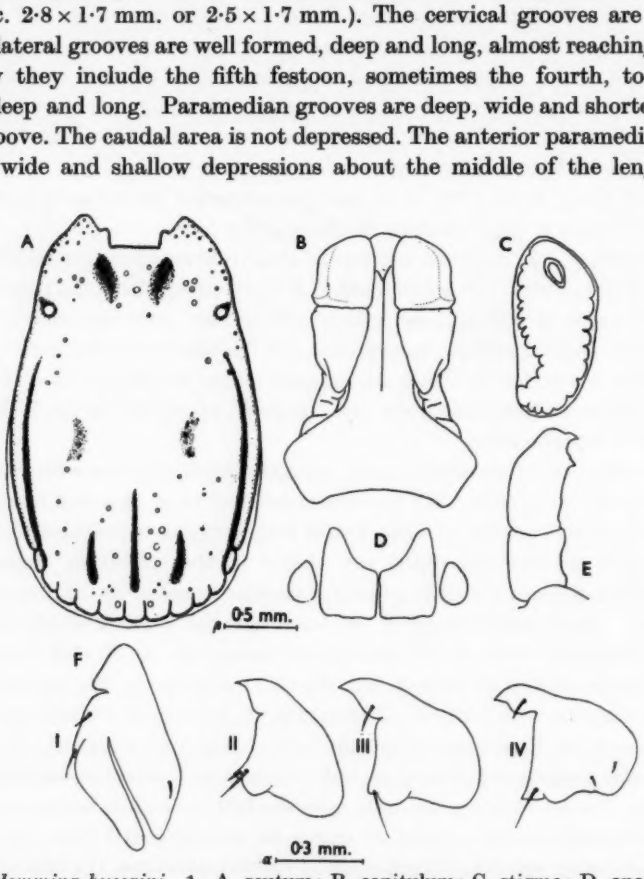

Fig. 6. *Hyalommina hussaini*, ♂. A, scutum; B, capitulum; C, stigma; D, anal armature; E, tarsus IV; F, coxae I, II, III and IV. Scale α refers to B, C, E and F; scale β to A and D.

scutum, and there are eleven well-separated festoons. The palps are longer than in *H. rhipicephaloides* (*c.* 0·65 mm.). The basis capituli is hexagonal, the lateral angles being blunt; the cornua small (Fig. 6B). The posterior internal and posterior external angles of the anal plates are right angles and the distal line is straight (Fig. 6D). The adanal plates are rounded distally. There are no subanal plates. The anal plates resemble those of *Hyalomma aegyptium* L. The tarsi are slightly humped (Fig. 6E). The first coxa is deeply divided, the two processes are about of the same length. There is a small spur on each of coxae II, III and IV, decreasing in size from coxa II to IV (Fig. 6F). The stigma has a very wide tail, with small and

numerous goblets, especially in the tail. The chitin girdle encircling the stigma is indented (Fig. 6C).

Female. The colour of the female resembles that of the male. The scutum is smooth and glossy and about as long as broad, with some large scattered punctations. The basis capituli is a broad hexagonal with lateral projecting angles, characteristic of the *Hyalommina*. The cornua are generally absent, but might be slightly indicated. The palps are long (*c.* 0·8 mm.) (Fig. 7A, B). Coxae as in the male. The stigma is very large with a wide tail and an indented chitinous ring with small and numerous goblets (Fig. 7C). The tarsi are slightly humped. The entrance into the female genital tube is not covered by an operculum. The upper cup-like portion of the mounted genital aperture is wide and rounded, the 'cup' being wider than deep (Fig. 8). This 'cup' can be seen in the unmounted tick.

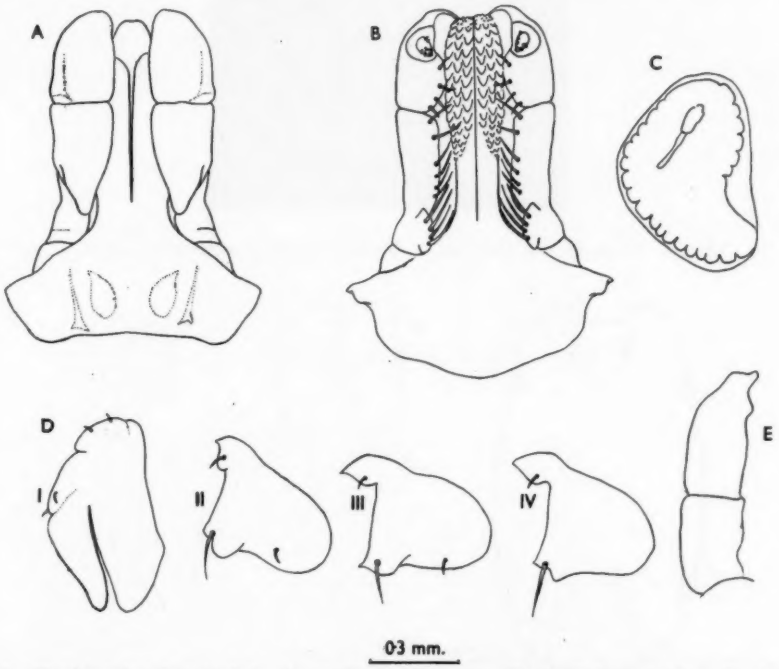

Fig. 7. *Hyalommina hussaini* ♀. A, capitulum dorsum; B, capitulum venter; C, stigma; D, coxae I, II, III and IV; E, tarsus IV.

Hyalommina kumari Sharif, 1928

Male. The scutum is normally pigmented. The colour of the scutum is light brown, the palps and the legs are somewhat lighter in colour. The anterior part of the scutum and the basis capituli are red-brown, the posterior part brown. The scutum is generally long and narrow (2·75 × 1·6 to 2·3 × 1·4 mm.), with some medium-sized punctations scattered over its anterior and posterior ends. The cervical and lateral grooves are very short. The median and paramedian grooves

are sometimes absent and sometimes faintly indicated. There are eleven well-separated festoons (Fig. 9A). The basis capituli is hexagonal, the lateral angles are somewhat more acute and more protruding than in *H. hussaini*. The palps are

Fig. 8. *Hyalommina hussaini* ♀. Mounted genital aperture. × 140.

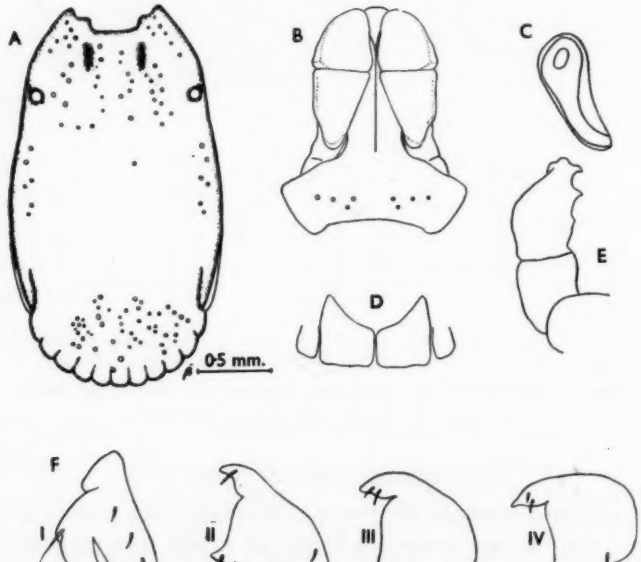

Fig. 9. *Hyalommina kumari* ♂. A, scutum; B, capitulum; C, stigma; D, anal armature; E, tarsus IV; F, coxae I, II, III and IV. Scale α refers to B, C, E and F; scale β to A and D.

somewhat wider than in *H. hussaini* and *c.* 0·5 mm. long. The third article of the palp is subtriangular (Fig. 9B). The median line of article II and III of the palps forms a less obtuse angle in *H. kumari* than in *H. hussaini*. There are small cornua. The anal armature resembles that of *H. hussaini*. The tarsi are strongly humped and stouter than in *H. hussaini* (Fig. 9E). The coxae are as in *H. hussaini*.

Sharif's description of the male allows for a quite wide variation of most characters. According to Sharif the lateral grooves are generally superficial and poorly developed, but may be absent in some cases. The postero-median groove is broad and shallow. The postero-lateral grooves (=paramedian grooves) are reduced to short oval depressions.

Female. The colour of the scutum is light brown. The scutum is slightly longer than broad (*c.* 1·7 × 1·8 mm.) with many small and large punctations. The basis capituli is a broad hexagon, with acute lateral projecting angles. There are no cornua. The palps are long (*c.* 0·8 mm.). The median line of the second and third articles of the palp forms a less obtuse angle than in *H. hussaini* (Fig. 10A). The chitinous ring of the stigma is as in other species of *Hyalomma* and not indented as

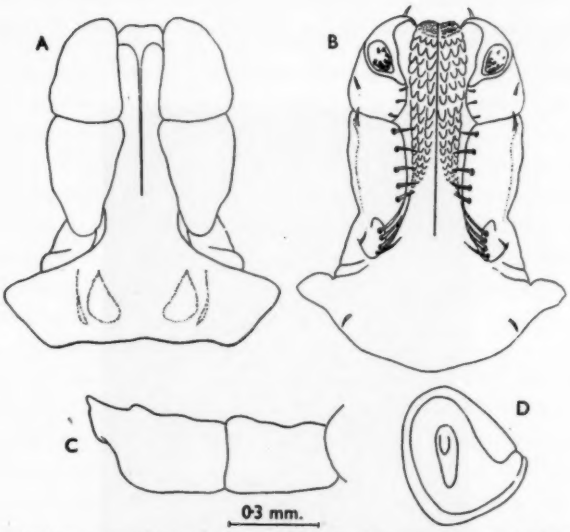

Fig. 10. *Hyalommina kumari* ♀. A, capitulum dorsum; B, capitulum venter; C, tarsus IV; D, stigma.

in *Hyalomma hussaini* (Fig. 10D). The tarsi are markedly humped (Fig. 10C) as in the male and stouter than in *H. hussaini*. The form of the mounted genital aperture is very characteristic. The operculum just reaches the entrance into the vaginal tube. The flaps of the cup-like portion of the genital tube are thick and not as pointed at their anterior end as in *H. hussaini*. The 'cup' is small and not as wide as in *H. hussaini* (Fig. 11). In the unmounted female the 'cup' of the genital aperture appears deeper than wide.

Discussion

In addition to the two quite distinct types of *H. hussaini* and *H. kumari*, described above, there are specimens which have intermediate characters between the two types. There are males with moderately humped tarsi and long lateral grooves as in *H. hussaini*, but with a punctated scutum and a stigma as in *H. kumari*. The corresponding females have also moderately humped tarsi as in *H. hussaini*, but a punctated scutum and an unindented stigma as in *H. kumari*. The genital aperture is microscopically as well as macroscopically the same as in *H. kumari*.

These specimens correspond to Sharif's description of *H. hussaini* var. *brevipunctata* and appear to be (also according to Sharif) intermediate between *H. kumari* and *H. hussaini*. They may also be included in the range of the individual variation of *H. kumari* as described by Sharif, with the only difference that the tarsi of these specimens are less humped than those of the typical *H. kumari*. According to Sharif's description, the difference between *H. kumari* and *H. hussaini* var. *brevipunctata* seems thus to be only in the degree to which the tarsi are humped. The question arises, therefore, whether *H. hussaini* var. *brevipunctata* constitutes a valid subspecies, or whether specimens described as such should be synonymized with one of the other Indian species of *Hyalommina*.

Fig. 11. *Hyalommina kumari* ♀. Mounted genital aperture. × 140.

In view of the striking variation known in other species of *Hyalomma*, *H. dromedarii* (Delpy, 1936), *H. excavatum* (Adler & Feldman-Muhsam, 1948), *H. truncatum* (Feldman-Muhsam, unpublished), it is impossible to make a definite statement on the status of these specimens, till the true range of variation has been determined experimentally by examining progeny of individual females.

Hyalommina lewisi Schulze, 1936 (*Species condemnanda*)

As mentioned above, Schulze considered specimens found in Kenya on 8 June 1935 on cattle as a new species of *Hyalommina* and called it *H. lewisi*. These specimens were collected together with specimens of *Hyalomma truncatum* K., 1844 (=*Hyalomma impressum transiens* Sch., 1919).

An examination of specimens of '*H. lewisi*' determined by Schulze showed that these ticks do not belong to the genus *Hyalommina*, but are, like the other specimens of the same lot, *Hyalomma truncatum*. The males of '*H. lewisi*' are not of the typical form of *H. truncatum*, but are within the limit of specific variation shown by laboratory breedings (kindly lent to us by Dr Gertrud Theiler). The females have the typical genital aperture of *H. truncatum* (Feldman-Muhsam, 1954). Neither the males nor the females have the characteristic humped tarsi of *Hyalommina*. The basis capituli is also as in other *Hyalomma* and not as in *Hyalommina*. The subanal plates are present in the specimens which we have examined. *H. lewisi* should therefore be synonymized with *Hyalomma truncatum*.

CONCLUSIONS

An examination of specimens of *Hyalommina rhipicephaloides*, *H. hussaini* and *H. kumari* shows very clearly that these are valid species and that there is no reason whatsoever to synonymize them with *Hyalomma excavatum* as Delpy has done (1946, p. 80, and 1949, p. 477).*

H. rhipicephaloides is a species of small size and is often poorly pigmented, but with constant characters which enable easy identification. The Indian species are somewhat larger in size, well pigmented, easily distinguished from other species of *Hyalomma* and form together with *H. rhipicephaloides* a well-defined group.

The features which characterize the subgenus *Hyalommina* are:

(1) Humped tarsi in males and females.

(2) Absence of subanal plates in the males.

(3) Basis capituli more or less hexagonal, with more obtuse and less protruding lateral angles in the males, and less obtuse and more protruding ones in the females.

As Neumann rightly observed with respect to *H. rhipicephaloides*, the specimens exhibit characters which are partly those of *Hyalomma* and partly those of *Rhipicephalus*. Neumann (1901, p. 318) says: 'Cette espèce...est intermédiaire à *Rhipicephalus* et *Hyalomma*. Elle a des premiers le facies général et la forme de pattes; elle se rattache aux seconds par le rostre, qui est, d'ailleurs, court et rappelle encore les *Rhipicephalus*.'

The Hyalomminas are linked to *Rhipicephalus* not only because the male of *H. rhipicephaloides* resembles *Rhipicephalus* in its general appearance, but also because of the absence of the subanal plates in the males and the laterally protruding angles of the basis capituli in the females.

* Taking for granted that *Hyalommina rhipicephaloides* is a synonym with *Hyalomma excavatum*, Dias (1955) considers the name *Hyalommina* inappropriate for the subgenus and proposes to change it into *Delpyiella*. But as *H. rhipicephaloides* is a valid species, *Delpyiella* Dias, 1955 is a synonym with *Hyalommina* Schulze, 1919.

The above species therefore constitute a link between *Hyalomma* and *Rhipicephalus*, and the problem arises whether the Hyalomminas should not be considered as a distinct genus. A study of the pre-imaginal stages might settle this problem. Unfortunately, we have not yet been able to study the pre-imaginal stages of the Indian species (and, as far as we know, no one else has described them), but the characters of the larva and nymph of *H. rhipicephaloides* show that this species is very similar in all its characters to other species of *Hyalomma* and is very closely related to them. We therefore think that *Hyalommina* is closer to *Hyalomma* than to *Rhipicephalus*, and should remain a subgenus of *Hyalomma*.

Key to the males of the subgenus Hyalommina

1. Anal plates rounded distally. Israel, Egypt *H. rhipicephaloides*.
— Angles at back of anal plates right angles: India 2
2. Red-brown specimens. Lateral grooves long, median and paramedian grooves present and well formed. Tarsi moderately humped **H. hussaini**
— Lateral grooves short or absent, median and paramedian grooves slightly indicated or absent. Tarsi strongly humped **H. kumari**

Key to females

1. Exterior process of 1st coxa longer than median process. Specimens slightly pigmented and of a pale colour (all three specimens known hitherto). Mounted genital aperture of the form of a deep rounded cup, with anteriorly pointed flaps. Small cornua present. Israel, Egypt **H. rhipicephaloides**
— The two processes of 1st coxa about the same length. Normally pigmented specimens. No cornua. India 2
2. Red-brown specimens. Tarsi slightly humped. Genital aperture wider than deep. Large stigma with indented chitinous ring **H. hussaini**
— Light brown specimens. Tarsi strongly humped. Genital aperture as wide as deep. Chitinous ring of stigma not indented **H. kumari**

SUMMARY

The validity of the subgenus *Hyalommina* is discussed and reaffirmed.

Hyalommina is differentiated from *Hyalomma* by the absence of the subanal plates in the males and by the humped tarsi and the more or less hexagonal basis capituli in both sexes.

The male and the female of *H. rhipicephaloides* Neumann, 1901 are re-described. The larva and nymph of *H. rhipicephaloides* are described for the first time.

The Indian species *H. hussaini* Sharif, 1928 and *H. kumari* Sharif, 1928 are re-described. *H. hussaini* var. *brevipunctata* Sharif, 1928 is considered as a *species inquirenda*.

H. lewisi Schulze, 1936 is synonymized with *Hyalomma truncatum* K., 1844 (Feldman-Muhsam, 1954).

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A COMPARATIVE STUDY OF THE INFECTIVE LARVAE OF *NEMATODIRUS* SPECIES PARASITIC IN SHEEP

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(With 6 Figures in the Text)

INTRODUCTION

Three species of the genus *Nematodirus* have been described from sheep in Britain: *N. spathiger*, *N. filicollis* and *N. battus*.

The infective larva of *N. spathiger* has been described by Monnig (1931), and in more detail by Dikmans & Andrews (1933). The infective larva of *N. filicollis* was described and figured by Boulenger (1915). A brief description was also given by Morgan (1930), while Maupas & Seurat (1913) figured the larva but gave no description or measurements. In the present paper, the infective larva of *N. battus* is described and compared with those of the other two species. However, as in the course of this study certain discrepancies were observed in the published description of the larva of *N. filicollis*, a redescription of the larva of this species is also given.

MATERIAL

Eggs of *N. battus* and *N. filicollis* were obtained by the dissection of fresh female worms collected from the intestines of sheep at slaughter, following the technique described by Barker (1946). Females of *N. battus* were identified on the character of the tail, which ends in a simple point (Crofton & Thomas, 1954). It is not possible to distinguish between the females of *N. filicollis* and *N. spathiger*, but in the present work only males of *N. battus* and *N. filicollis* were found in association with these females and no males of *N. spathiger* were recovered. It was therefore assumed that only females of *N. battus* and *N. filicollis* were present.

In addition, it is possible to distinguish between the eggs of *N. filicollis* and *N. spathiger* on morphology and size measurements. The eggs of these two species have been compared by Tetley (1941) and by Kates & Shorb (1943), who state that the eggs of *N. spathiger* may be identified by their larger size and by the presence of polar thickenings of the shell. Measurements given by these and other authors, together with those obtained in the present work, are shown in Table 1. Measurements of *N. filicollis* eggs given by Boulenger (1915) are also included and are seen to cover a much greater range. The measurements obtained in the present work agree very closely with those of Tetley (1941). All *N. filicollis* eggs used in this study were carefully identified by morphological examination and size measurements. Several hundred worms were examined during this work, but no males of *N. spathiger* were recovered and only one female worm was assigned to this species on the basis of egg characteristics. It would appear, therefore, that *N. spathiger*

is uncommon in north-east England, the area from which the slaughterhouse material was obtained.

As females of *N. spathiger* were not available, eggs of this species were obtained by flotation from faeces samples collected in the south where *N. spathiger* appears to be more prevalent, and were identified by morphology and size measurements as described above.

Table 1. *Egg measurements in* μ

		Tetley (1941)	Kates & Shorb (1943)	Present work	Boulenger (1915)	Railliet & Henry (1912)	May (1920)
N. filicollis	Length	140–165	149–194	134–168	130–210	145–180	130–200
	Max. diameter	70–85	74–107	71–87	65–95	75–90	70–90
N. spathiger	Length	180–210	181–230	183–214	—	200–260	150–220
	Max. diameter	90–105	91–107	87–99	—	100–110	80–110
N. battus	Length	—	—	160–189	—	—	—
	Max. diameter	—	—	67–77	—	—	—

Only eggs in the 7–8-cell stage were taken, and were cultured at 21° in Petri dishes in a depth of a few millimetres of water. The eggs are ready to hatch only when the third larval stage is reached, this taking approximately 18–22 days in the case of *N. spathiger* and 26–30 days for *N. filicollis* and *N. battus*.

Specimens for measurement and drawing were killed by gentle heating and mounted in water under a raised cover-slip. Measurements were made with an ocular micrometer scale, and drawings were made with the aid of a camera lucida.

Nematodirus spathiger: *infective larva*

The larva, which is enclosed in the second-stage sheath, is from 976 to 1130 μ long and possesses a long sheath tail, characteristic of the genus. The buccal cavity is simple and opens into the oesophagus which is 192–224 μ long. The intestine is composed of eight elongated cells lying alternately dorsal and ventral, and terminates in a slender rectum, opening at the anus, 64–74 μ from the tip of the tail. The larval tail is forked and between the dorsal and ventral lobes lies a digitate terminal process, 15 μ long. The sheath tail is long and slender, the distance from the anus to the tip of the sheath being from 310 to 390 μ.

The larva has been described and figured by Monnig (1931) and by Dikmans & Andrews (1933), both authors describing the larva as possessing a bifid tail with a rod-shaped terminal process. In the present work, only a small number of larvae of *N. spathiger* were available, but these were found to correspond closely with the descriptions given by the above authors. Measurements made in this work agree very closely with those of Dikmans & Andrews (1933) (Table 2).

Nematodirus filicollis: *infective larva*

The larva generally resembles that of *N. spathiger*. It is enclosed in the second larval sheath, the first larval sheath being cast and remaining within the shell at hatching. The overall length is 752–1018 μ, this larva being the smallest of the

three species considered. The buccal cavity is a simple tube leading into a filariform oesophagus, 170–210 μ long. The intestine is very prominent, consisting of eight elongated cells lying alternately dorsal and ventral, the cells being filled with dense granular material. The genital primordium is from 304 to 416 μ from the anterior end, towards the ventral surface, at the level of the fifth intestinal cell. The intestine narrows abruptly, terminating in a slender rectum opening 32–48 μ from the posterior end of the body. In lateral view the larval tail ends bluntly in

Table 2. *Measurements in μ of infective larva*

	N. spathiger		
	Monnig (1931)	Dikmans & Andrews (1933)	Present work
Length of sheath	1158 (1119–1177)	1009 (922–1118)	1018 (976–1130)
Length of larva	877	741	751 (723–810)
Length of oesophagus	235 (227–235)	195 (160–225)	204 (192–224)
Anterior end–genital primordium	509	417 (365–500)	428 (394–480)
Larval tail	59 (43–59)	60 (52–65)	69 (64–74)
Anus–tip of sheath	340 (325–340)	328 (315–350)	332 (310–390)
Caudal process	—	10–15	15

	N. filicollis		
	Boulenger (1915)*	Maupas & Seurat* (1913)	Present work
Length of sheath	1075	770	912 (752–1018)
Length of larva	790	560	600 (496–672)
Length of oesophagus	214	150	193 (170–210)
Anterior end–genital primordium	474	312	369 (304–416)
Larval tail	57	37	39 (32–48)
Anus–tip of sheath	342	247	370 (294–406)
Caudal process	10	Absent	Absent

N. battus	
Length of sheath	1026 (963–1136)
Length of larva	855 (774–928)
Length of oesophagus	223 (205–246)
Anterior end–genital primordium	486 (442–550)
Larval tail	88 (70–96)
Anus–tip of sheath	259 (211–313)

* Measurements made on authors' drawings.

a deep notch which divides it into a stout ventral lobe and a slightly smaller dorsal lobe. The ventral lobe is further subdivided by a midline cleft into two ventro-lateral lobes, thus making the tail tricuspid. The tail differs from that of *N. spathiger* in that there is no rod-shaped process lying between the dorsal and ventral lobes. The sheath tail is long and finely attenuated, the distance from the anus to the tip of the tail varying from 294 to 406 μ.

The description of the larva of *N. filicollis* given by Boulenger (1915) differs from the above, both in the overall measurements and in the character of the tail of the larva. The measurements given by this author are considerably larger and agree more closely with the measurements of *N. spathiger* obtained in the present work than with those for *N. filicollis*. Dikmans & Andrews (1933) noted that their measurements for *N. spathiger* agreed closely with those of Boulenger (1915) for

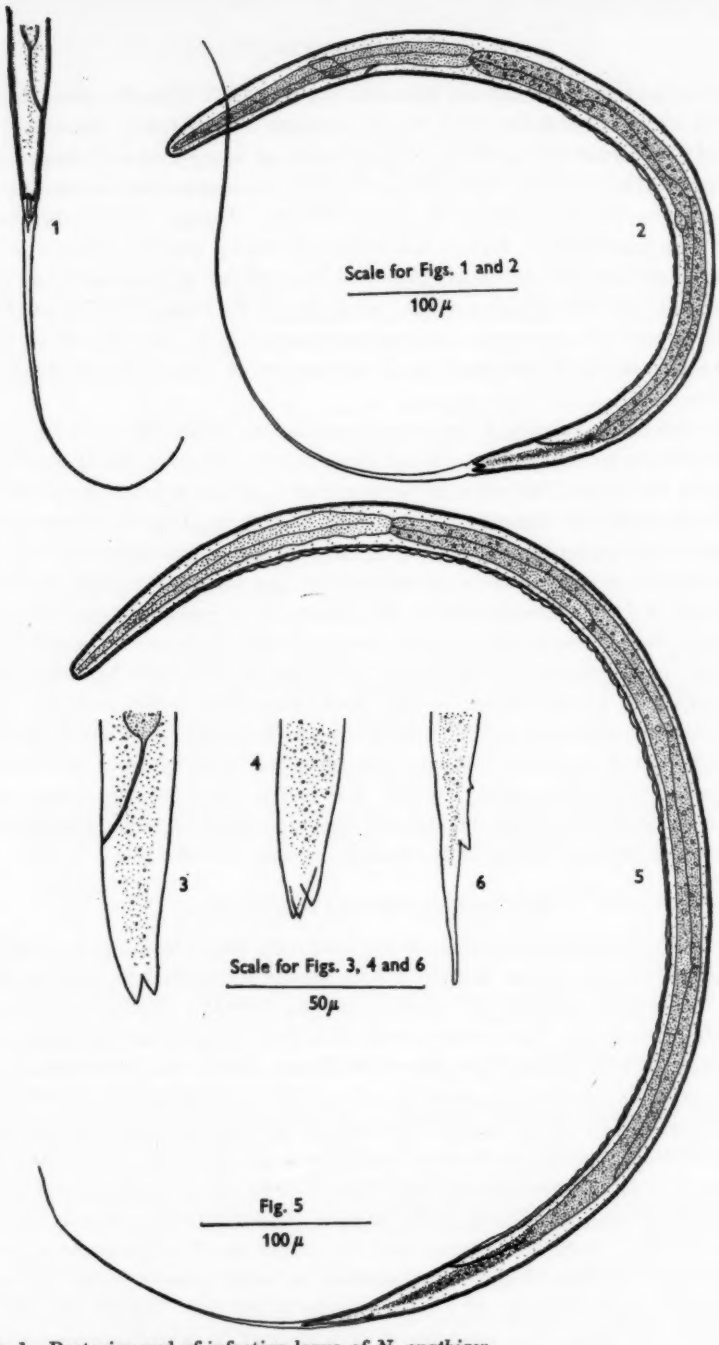

Fig. 1. Posterior end of infective larva of *N. spathiger*.
Fig. 2. Infective larva of *N. filicollis*.
Fig. 3. Posterior end of exsheathed infective larva of *N. filicollis*, lateral view.
Fig. 4. Posterior end of exsheathed infective larva of *N. filicollis*, ventro-lateral view.
Fig. 5. Infective larva of *N. battus*.
Fig. 6. Posterior end of exsheathed infective larva of *N. battus*, lateral view.

N. filicollis, and also pointed out that the drawing of *N. filicollis* larva figured by Maupas & Seurat (1913) was considerably smaller. In addition, Boulenger (1915) described the larval tail as being forked, with an elongated rod-shaped process projecting well beyond the lobes of the tail, this description corresponding closely with that given for *N. spathiger* by other workers. Morgan (1930) also described the larva as possessing a forked tail with a terminal process. Cameron (1923), however, mentions the larva of '*Nematodirus* species' as possessing a 'peculiar tricuspid tail'. In the drawing of this larva figured by Maupas & Seurat (1913) no terminal process is apparent, and measurements made on this drawing agree more closely with those obtained in the present work than with those given by Boulenger (1915).

During the present study, a large number of larvae of this species were examined carefully for the presence of a terminal process but in no case was it observed. In addition, it was found that after immersion in 2 % pepsin solution at pH 3–5 and at 37°, the second sheath was cast. These exsheathed larvae (Figs. 3, 4) were examined in detail for the presence of a terminal spike, but again this character was absent.

On this evidence it is therefore considered that the description given by Boulenger (1915) was, in fact, a description of the larva of *N. spathiger* and not that of *N. filicollis*. Table 1 shows that the egg measurements given by Boulenger (1915) for *N. filicollis* considerably overlap those given for *N. spathiger* by other workers. As the eggs used by the former author were taken from faeces samples, it would appear probable that this author was dealing with mixed cultures of eggs of both *N. filicollis* and *N. spathiger*. It has been observed in the present study that eggs of *N. spathiger* develop more rapidly and hatch more readily than those of *N. filicollis*. Thus from a mixed culture of eggs of these two species, a relatively high proportion of hatched larvae of *N. spathiger* would be obtained.

Nematodirus battus: *infective larva*

The larva is typical of the genus, being relatively large, from 963 to 1136 μ long. It is enclosed in the rather loosely fitting second-stage cuticle, with a long tail sheath. Anteriorly, no lips are visible, but six cephalic papillae are present as small refractive spots. The buccal cavity is a short simple tube opening into the oesophagus, 205–246 μ long, the cells of which are rather transparent and difficult to make out. The excretory canal is a fine tube opening at the excretory pore approximately 170 μ from the anterior end, on the ventral surface. Just anterior to the excretory pore the oesophagus passes through the nerve ring. The intestine is composed of eight alternate cells and narrows to a slender rectum, opening from 70 to 96 μ from the tip of the tail. The genital primordium lies towards the ventral surface, 442–550 μ from the anterior end. The tail of the larva tapers to a fine point, and bears two notches on the dorsal surface, a small anterior notch and a larger posterior one approximately 37 μ from the tip of the tail. The sheath tail is long and slender but rather less attenuated than that of *N. filicollis*, measuring from 211 to 313 μ from the anus to the tip of the sheath.

The larva of *N. battus* has not been previously described. It is of approximately the same dimensions as that of *N. spathiger*, but differs markedly from the other

two species in the shape of the larval tail. The differences between the larvae would appear to be correlated with differences between the adult females, for both the adult and larva of *N. battus* are characterized by the finely pointed tail, whereas in the adult and larval stages of *N. spathiger* and *N. filicollis*, the tail ends bluntly.

CONCLUSION

The infective larvae of *N. spathiger*, *N. filicollis* and *N. battus* are very similar morphologically, differing only in size, and in the character of the tail. On these differences the differentiation of the larvae may be based. The larva of *N. filicollis* is markedly smaller than those of the other two species, but the difference in length is masked to some extent by the very long sheath tail of the former. The larvae may be most easily identified on the shape of the larval tail, which is usually visible under low-power magnification, particularly in the differentiation of *N. battus* and *N. filicollis*. Some difficulty may be found in the differentiation of *N. filicollis* and *N. spathiger*, as in both species the tail is forked, and it is difficult in some positions to determine whether the terminal process, characteristic of *N. spathiger*, is present or absent. In such cases measurements of larval length and sheath tail length are of value in making an accurate identification.

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ELECTRON MICROSCOPIC OBSERVATIONS OF *TOXOPLASMA* 'NICOLLE ET MANCEAUX' IN THIN SECTIONS OF TISSUE CULTURES

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(With Plates III–VIII)

In a previous paper (Meyer & Mendonça, 1955) we gave results of our study of *Toxoplasma* (Nicolle et Manceaux) carried out within the electron microscope, on the parasites in unsectioned preparations of tissue cultures. We have shown that the whole body of *Toxoplasma* is covered by a very delicate, transparent mantle devoid of special structure and extending to form large membranes. The rest of the body in these *in toto* preparations was too thick for a sufficient penetration of the electron beam to reveal the inner structure.

Thin sections of the same material have now been prepared and examined in the electron microscope, and the results of this investigation will be discussed in this paper.

Thin sections of *Toxoplasma*, obtained from the peritoneal exudate of infected mice and of minced liver tissues, have been studied recently by Gustavson, Agar & Cramer (1954), who described some longitudinal inclusions, peculiar to this parasite, in connexion with a mouth- or cone-like formation at one end of the body as well as lipid inclusions, fine granules in the region of the centriole, mitochondria and fragments of an endoplasmic reticulum.

MATERIAL AND TECHNIQUE

As in our previous work *Toxoplasma* was cultivated in subcutaneous and muscle tissues from 12- to 14-day-old chick embryos. When the cultures showed the desired aspect they were fixed for 10–20 min. in 1 % osmic acid, buffered at pH 7·6–7·8 with veronal acetate. Fixation, washing and dehydration in alcohol was done on the slides supporting the hanging-drop cultures. Only in the 90 % alcohol, when the cultures had greater consistency, were they cut out, and the procedure was continued as with any piece of tissue. For the embedding, *n*-butyl methacrylate was used, and the embedded cultures were cut with the Porter–Blum microtome in sections which, according to their reflexion colour, ranged from 40 to 80 mμ in thickness. They were examined with the 'Philips' electron microscope (80 kV.).

RESULTS

In highly infected tissue cultures we usually find at the site of the intracellular forms, parasites which had just left the host cells as well as many degenerated and degenerating forms of *Toxoplasma*. Great masses of cell debris are always present, and the parasitized as well as the unparasitized cells, adjacent to a much infected

area, show signs of degeneration such as vacuoles and lipid granules. During the present investigation our attention has been focused especially on the intact intracellular forms of *Toxoplasma*, single and in rosette formation, and on those extracellular parasites which showed no alteration in their structure.

Figs. 1–4* show rosette formations of *Toxoplasma* inside the cell. Two different poles can be easily distinguished in the parasite, the central one and the one at the periphery of the rosette. The parasites of one rosette are not cut all at the same level, so that different images of the single forms are obtained. We see a ring-like formation at the peripheral end of the body and, in accordance with the authors Gustavson *et al.*, long, dark homogeneous inclusions connected with this formation. These inclusions, the number of which varies with the parasite, are thinner at the peripheral end, the longest of them reaching down to the nuclear region and sometimes branching. They seem to be solid and rather resistant elements because they are as dark and homogeneous in transverse as in the longitudinal sections, and bundles of them are often found among the debris of degenerated areas.

The nucleus of *Toxoplasma* shows a distinct membrane, a nucleolus and often some chromatine granules. No mitotic or other dividing figures have been found so far with certainty.

The surface of the parasite shows a double membrane which is especially strong at the two poles, at the ring-like formation of the peripheral end which often slightly protrudes (Figs. 1–5, 8), and at the opposite pole where a distinct opening can be observed in many sections (Fig. 3). The membrane is never as dark or as well defined in the region between the two poles. Fragments of a fine canal system about Å400. wide, similar to the endoplasmic reticulum described by Palade & Porter (1954) in cells and minute mitochondria, can be seen in the cytoplasm (Figs. 2, 4, 7); also small granules, probably of lipid nature, and very often a group of slender striae or tubules, approximately 250 Å. wide, are found near the nucleus in the region of what may be the centrosome and the Golgi apparatus (Fig. 2). In some sections these have the aspect of a spiral, and below them, adjacent to the nucleus, can be seen some very fine granules, surrounded by a circular area (Fig. 4).

The ground substance is represented by fine granules of varying size 250–400 Å. in diameter, which, according to the direction of the section, may appear as irregular striae (Figs. 2, 4). In a few micrographs of apparently very superficial, tangential sections a fine striation can be seen which might belong to a thin periplast of the parasite (Fig. 6).

The cytoplasm of the host cell surrounding a group of parasites is always dissolved. A slight thickening but no distinct wall of the vacuole can be observed in the cell's cytoplasm (Figs. 1, 3). Also the single forms are surrounded by a lighter area which probably indicates the beginning of cytolysis (Fig. 9). The nuclear membranes of the host cells as well as the membranes of the mitochondria resist the lysing action of the parasite (Figs. 1–4 and 7). The host cell's mitochondria near a group of parasites always show alterations. They seem to be swollen, and the typical cristae, described by Palade (1952), are faded or completely destroyed (Figs. 1–4).

* Pls. III–VIII contain figs. 1–9.

DISCUSSION

The images which have been obtained of *Toxoplasma* during this investigation clearly show the absence of cilia, flagella or other organelles for locomotion. The fine membranes which have been observed in the unsectioned parasites do not appear in the intracellular forms; however, there is no doubt that the surface membrane between the two poles is much thinner and less defined than at the poles and probably is able to extend. It is probably not strong enough to maintain the shape of the parasite and to protect it; this explains the deformed and degenerated *Toxoplasma* found outside the tissue cells.

Comparing the parasite in the rosette with that of the single form we see that the difference in size between the two poles is much less pronounced in the latter. The host cells in tissue cultures are often greatly flattened, and the parasites inside the cells flatten with them. To a certain extent they are able to adapt their form to the environmental conditions and are much larger at the periphery of the rosette than at the central pole.

Since *Toxoplasma* lives and reproduces exclusively inside the cells, it is not very likely that the two pole structures are mouth formations. The parasites probably take in food through the thin membrane of their body. In most of our sections the membrane which runs over the peripheral pole is closed. It is thinner though, and a small process is often attached to it (Fig. 8). From observation *in vivo* we know that *Toxoplasma* penetrates the host cells with this part of the body. It seems more likely to us that these formations are destined to make parasitism possible. Something is probably released by the parasite which helps penetration, dissolving the cell's cytoplasm, thus diminishing its resistance, and later preparing space for the increasing number of parasites in the cell. It is also possible that the cytoplasm has to be digested into a more liquid form in which it is more easily assimilated by the parasite. We do not know the role and the significance of the long inclusions which are found in connexion with the ring-like formation at the peripheral pole.

So far we have failed to find parasites in the act of division. From the direct observation of *Toxoplasma* in living tissue cultures we know that it divides by binary fission and that the process is very rapid. We have not been able yet to see in the electron micrographs how the multiplication of the nucleus and of the inclusions of the cytoplasm takes place.

SUMMARY

Toxoplasma 'Nicolle et Manceaux' has been examined in the electron microscope in thin sections of infected tissue cultures. The intracellular forms, single or in rosette formations, show that the parasite has no flagella, cilia or other organelles for locomotion. It is covered by a double membrane which is especially well defined at the two poles. A ring-like formation, often slightly protruding, can be seen at one end of the parasite, and in connexion with this, long, homogeneous, dark-stained inclusions of still unknown nature. At the opposite pole a distinct opening has been found.

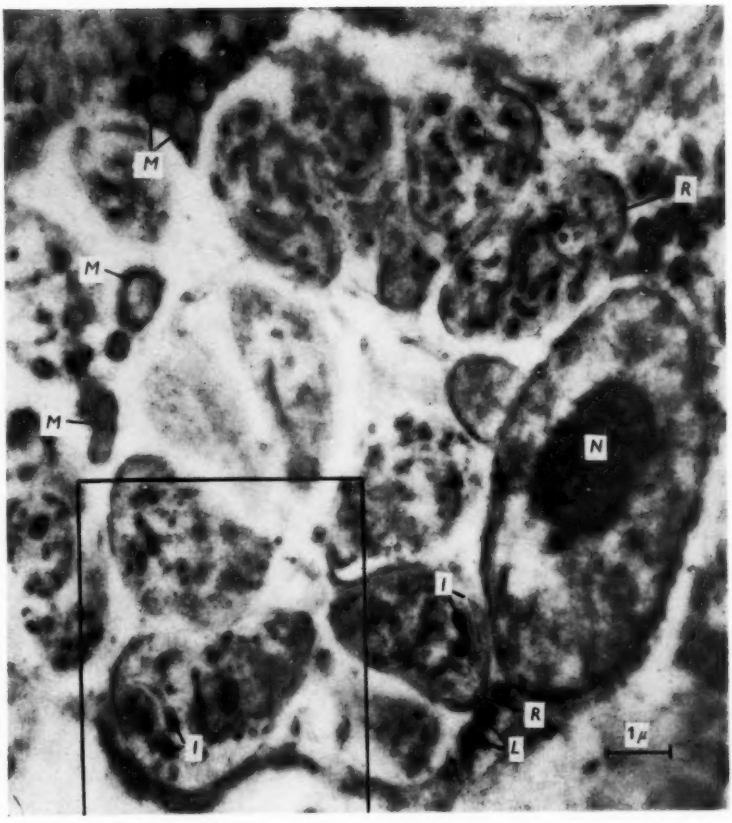

Fig. 1

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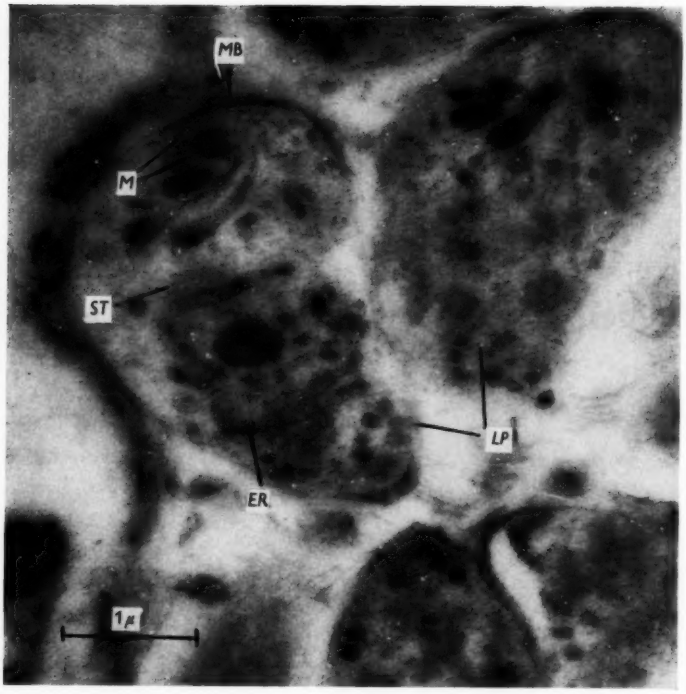

Fig. 2

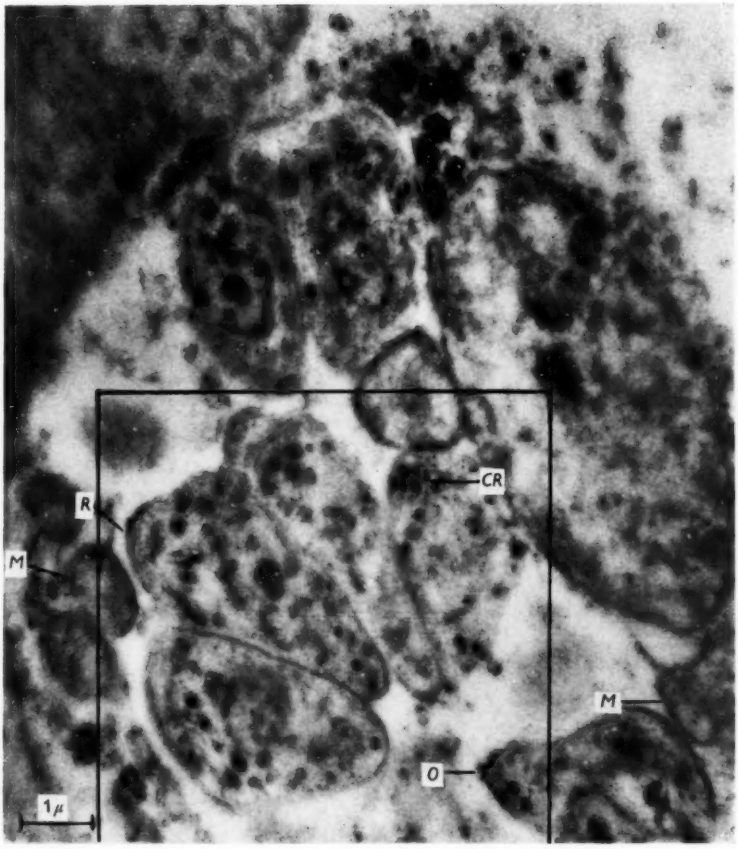

Fig. 3

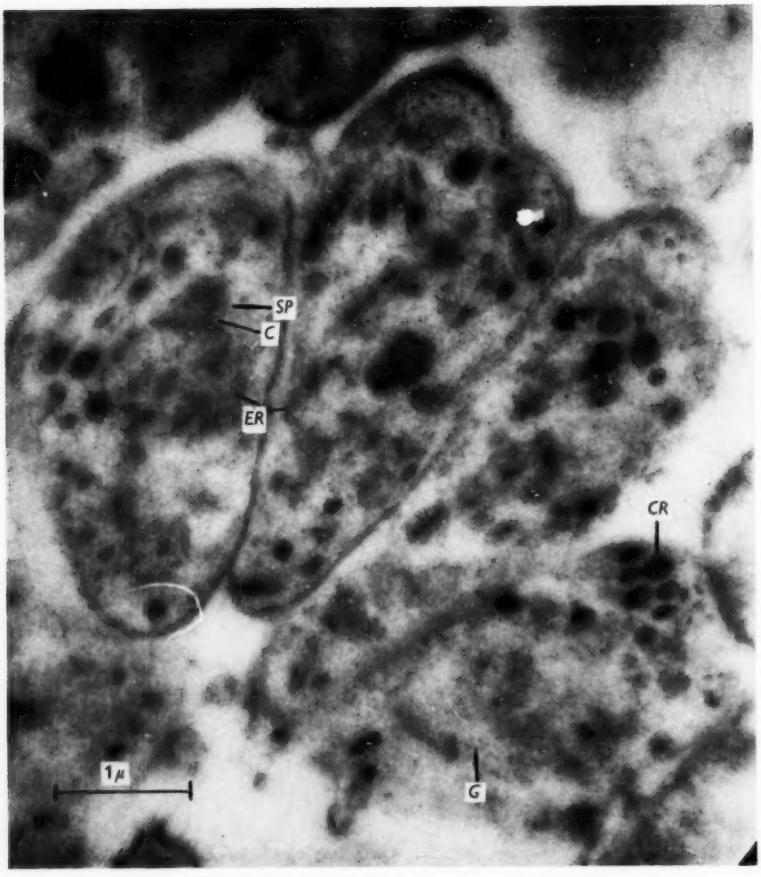

Fig. 4

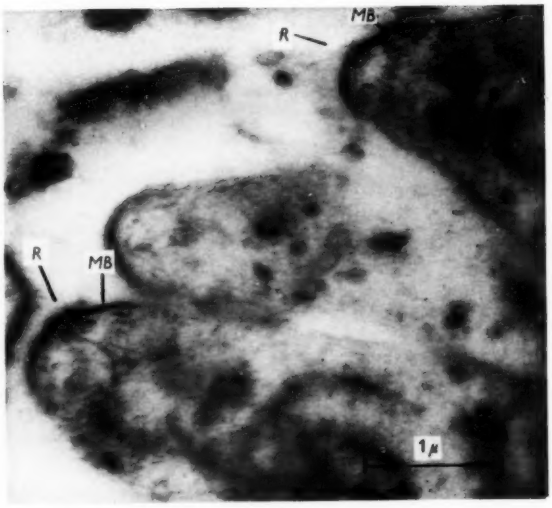

Fig. 5

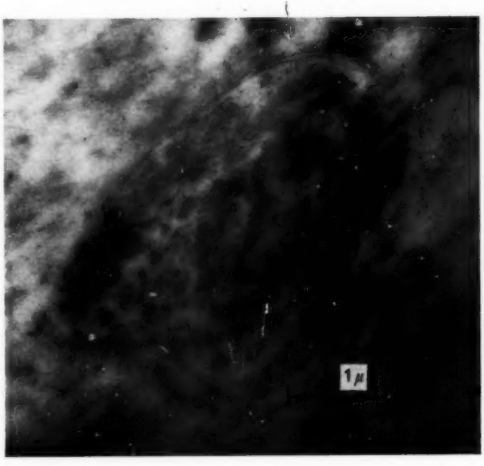

Fig. 6

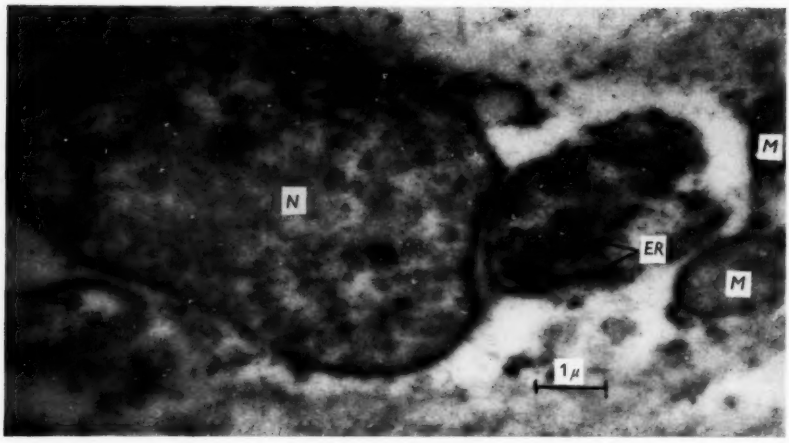

Fig. 7

Fig. 8

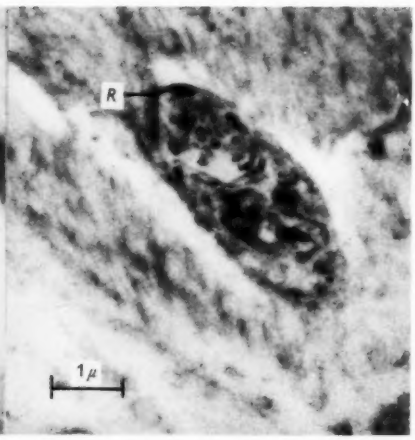

Fig. 9

Mitochondria and strands of an endoplasmic reticulum are also present in the cytoplasm and a fine striation in the region of the periplast. No dividing forms have been observed. Near the nucleus, in the region of what may correspond to the centrosome and the Golgi apparatus, a few extremely fine granules and a spiral-like formation of fine striae or tubules have been observed.

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EXPLANATION OF PLATES III–VIII

Plate III

Fig. 1. Rosette formation of *Toxoplasma* inside cell. Cytoplasm around parasites dissolved. *N*-nucleus of host cell with intact membrane. *L*, lipid granules. *M*, mitochondria of host cell with intact membranes but altered structure. *I*, long, homogeneous inclusions at peripheral pole of parasite. *R*, ring-like formation at peripheral region of parasite, slightly protruding. × 9000.

Plate IV

Fig. 2. Higher magnification of rectangular space in Fig. 1. *LP*, lipid granules. *ER*, endoplasmic reticulum. *St*, fine striae or tubules in Golgi region. *M*, mitochondria. *MB*, double membrane. × 20,000.

Plate V

Fig. 3. Rosette formation of *Toxoplasma* inside cell. *R*, ring-like formation at peripheral region. *O*, opening at central pole. *CR*, cross-sections of inclusions. *M*, mitochondria of host cell. × 10,000.

Plate VI

Fig. 4. Higher magnification of rectangular space in Fig. 3. *ER*, endoplasmic reticulum. *C*, fine granules in centrosome-area. *SP*, spiral formation in Golgi region. *CR*, cross-sections of long inclusions. *G*, ground substance. × 20,000.

Plate VII

Fig. 5. Peripheral regions of three parasites in rosette. *MB*, double membrane at poles more distinct than at sides. *R*, ring-like formation. × 20,000.

Fig. 6. Single parasite inside cell. Very superficial, tangential cut through only part of parasite. Fine striation in periplast. × 20,000.

Plate VIII

Fig. 7. Single form of *Toxoplasma* inside cell. Only part of body in cutting plane. Cytoplasm around parasite shows beginning of cytolysis. Nuclear membrane and membranes of host cell's mitochondria resist. Nucleus of cell is pushed back by parasite. *N*, nucleus of cell. *M*, mitochondria of cell. *ER*, endoplasmic reticulum of parasite. × 10,000.

Fig. 8. Part of rosette inside cell. *PR*, fine process attached to membrane at peripheral pole of parasite. × 13,335.

Fig. 9. Single form of *Toxoplasma* inside cell. Ring-like formation at one pole, slightly protruding, with dark, well-defined double membrane in this region. Cytoplasm of host cell shows beginning of lysis around parasite. × 10,000.

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STUDIES ON PHOSPHATASE SYSTEMS OF CESTODES

I. STUDIES ON *TAENIA PISIFORMIS* (CYSTICERCUS AND ADULT)

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(With Plate IX and 4 Figures in the Text)

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INTRODUCTION

Although the number of studies in the field of cestode enzymology is rapidly increasing, relatively little work has been carried out on phosphatase enzyme systems. Read (1949, 1950, 1951) has demonstrated the active uptake of phosphorus by the cestode *Hymenolepis diminuta* and shown that carbohydrate degradation occurs along enzymatic paths similar to those described in mammalian muscle, thus implying the presence of the associated phosphatases. Biochemical studies with tissue extracts of *Taenia pisiformis* have been carried out by Pennoit-De Cooman & Van Grembergen (1947), who recorded alkaline phosphatase activity in the adult tapeworm and acid phosphatase activity in the cysticercus, postulating a complete change-over when the cysticercus enters the final host. The only recorded pH-activity curves of cestode phosphatase are those published by Von Brand (1952) and constructed from the figures obtained by Pennoit-De Cooman & Van Grembergen. In addition, Read (1949) has demonstrated an alkaline phosphatase with an optimum pH 7·6 in extracts of *Hymenolepis diminuta*. The location of phosphatases has been studied by Rogers (1947), who, using the cobalt sulphide method of Glick, demonstrated the presence of alkaline phosphatase in the cuticle of *Moniezia expansa*.

As no combined histochemical and biochemical study has been made in this field, the following experiments were undertaken in order to obtain more detailed

information concerning the phosphatases present in both the adult and cysticercus of *Taenia pisiformis*. Particular stress was given to the construction of pH-activity curves and the effect on these of various inhibitors, thus allowing a more detailed comparison to be made between the phosphatase activity exhibited by the adult and cysticercus. It must be emphasized that the procedures involved allowed only the relative values of enzyme activity at varying pH's on the various experiments to be compared. The crude, aqueous extracts used did not provide information as to the absolute values of activity, and, from this point of view, the experiments are not comparable. In addition, histochemical studies provided information on the localization of the enzymes, thus facilitating interpretation of their possible function.

MATERIAL

Adult tapeworms occur in the intestine of the dog and the cysticercus stage in the abdominal cavity of the rabbit. Laboratory infections of both stages were maintained in experimental animals, thus enabling material of a known age to be used in the preparations of extracts. In the case of adult tapeworms the laboratory supply was supplemented by material from outside sources. In such cases the age of the material could not be determined. Forty-one dogs from various sources were examined and of these eleven were infected with *T. pisiformis*, giving an infection rate of about 27 %.

METHODS

(*a*) *Histochemical*

After removal from the host the parasites were washed in warm saline and then fixed in absolute acetone at 4°. The histochemical procedure of Glick (1949) using sodium glycerophosphate as substrate was followed. Incubation time was 1 hr. at 37° in the alkaline substrate and 3 hr. at 37° in the acid substrate. This latter incubation time was determined after diffusion tests similar to those performed by Martin & Jacoby (1949). Sections were incubated for 12 and 24 hr. in the case of doubtful positive and apparent negative reactions. Controls were of two kinds. In one, sections denatured by placing in boiling distilled water for 2 min. were incubated in normal substrate. The other control consisted of incubating sections in a mixture in which the glycerophosphate solution had been replaced by distilled water. Bacterial contamination was reduced by using toluene-saturated substrates and buffers and by covering all incubation mixtures with a thin toluene layer. Sections were generally cut in the horizontal plane, parallel to the long axis of the body, at a thickness of 10 μ. This plane allowed a greater variety of tissues to be tested per section than in the transverse sections which were also used. Each adult tapeworm was cut into short lengths containing 2–5 proglottides. The sections from each length were divided into batches for use as controls, for incubation in acid substrate, in alkaline substrate and for staining in Ehrlich's haematoxylin. In this way every region of each worm was tested, thus enabling a given phosphatase reaction to be related to a proglottis of known degree of development.

The distribution of glycogen was studied in horizontal sections of Carnoy-fixed material using the techniques of Best and of Gomori (Glick, 1949). Control sections were incubated in diluted saliva for 3 hr. at 37°.

(*b*) *Biochemical*

Enzyme activity was studied using aqueous, non-buffered extracts of fresh material. The tissues were ground up in distilled water with sand giving a suspension which was then saturated with toluene and stored at room temperature for a few hours. This mixture was centrifuged and the supernatant fluid so obtained removed and diluted to the required volume. The final mixture was stored under toluene in the refrigerator. In addition, all substrates and buffers were saturated with toluene before use.

The substrate used was disodium phenyl phosphate (M/100) and the phenol released by enzyme action was estimated colorimetrically by the method of Folin and Ciocalteau (Milton & Waters, 1949). Incubation mixtures consisted of 1 ml. buffer, 1 ml. substrate, 0·5 ml. extract and 0·5 ml. of toluene. All mixtures were incubated at 37°. The buffers used were the citrate and borate buffers of Sørensen (Clark, 1922), and the pH of the incubation mixtures was determined electrometrically with a glass electrode before and after incubation. At the end of the incubation period 1 ml. samples were removed for estimation. The inhibitors sodium fluoride and potassium cyanide were dissolved in the buffer in sufficient quantity to give a final concentration of M/100 in the incubation mixture. Controls of two kinds were employed. In one, heat-denatured extract was used and in the other the enzyme extract was replaced by distilled water. In all other details the control incubation mixtures were similar to the corresponding experimental series.

RESULTS

(*a*) *Histochemical studies*

(1) *The distribution of acid and alkaline phosphatases*

Cysticercus. In spite of repeated efforts, all attempts at the demonstration of both acid and alkaline phosphatases were unsuccessful.

Young tapeworms, 50 days old. In the course of experimental infections a batch of worms, 50 days old, was obtained. Acid phosphatase tests were negative except for a doubtful positive in the anterior region of some of the larger worms. The reaction occurred at the base of the cuticle. Alkaline phosphatase tests were positive and the reaction was confined to the base of the cuticle. No reaction was observed in the scolex.

Adult tapeworms. Acid phosphatase tests indicated the presence of an enzyme in the cuticle but with an uneven distribution along the length of the worm. Consistent, well-defined reactions occurred in the cuticle of the mature proglottis. Anteriorly and posteriorly to this region the intensity of the reaction diminished and became less consistent in occurrence. Ripe proglottides, which had been shed, were completely negative. Although the acid phosphatase reaction was confined

to the cuticle, the picture presented in this region depended on the time of incubation. When the incubation time was short the reaction was confined to the base of the narrow channels present in the cuticle. Increasing the period of incubation resulted in the expansion of the reaction outwards along the base of the cuticle. Further incubation resulted in the extension of the reaction from the proximal to the distal portion of the cuticle. Times for this diffusion of the reaction cannot be given, as the precise speed differed with the different blocks tested. In addition, it frequently happened that while one side of a horizontal section gave a continuous reaction along the entire cuticle, the other side exhibited reactions confined to the bases of cuticular channels (see Pl. IX, figs. 1, 2).

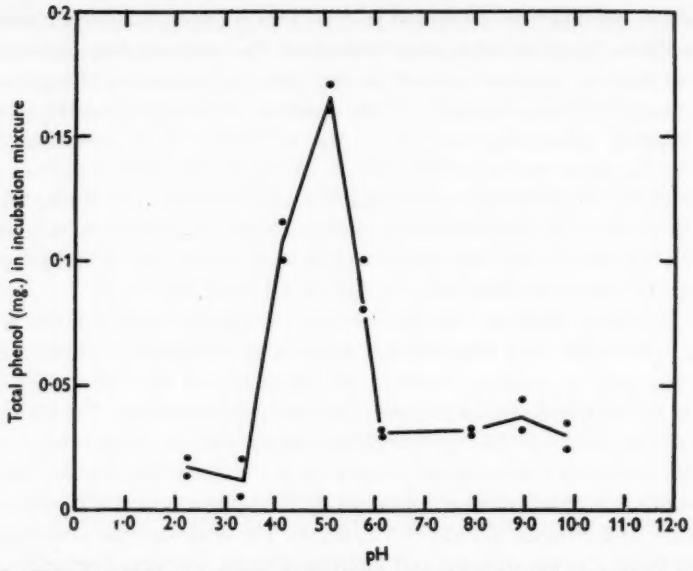
Unlike the acid enzyme, the distribution of alkaline phosphatase was more extensive. The scolex was negative but the rest of the worm, including free, ripe proglottides, gave a positive reaction. The intensity of the result seemed to be greatest in the region of almost mature and mature proglottides. The main reaction occurred in the cuticle (Pl. IX, fig. 3) with subsidiary location in the subcuticular cells and in the cuticular lining of the vagina of the mature proglottis. The uneven linear distribution was further emphasized by the appearance of a positive reaction in the ovary and vitelline gland (Pl. IX, fig. 4). The reaction was initially given by the tunica bounding the ovarian and vitelline lobules, and this gradually extended into the nuclei and cytoplasm of the cells. The effect of variation of incubation times was similar to the results quoted for acid phosphatase.

(2) *Histochemical characteristics of the phosphatases*

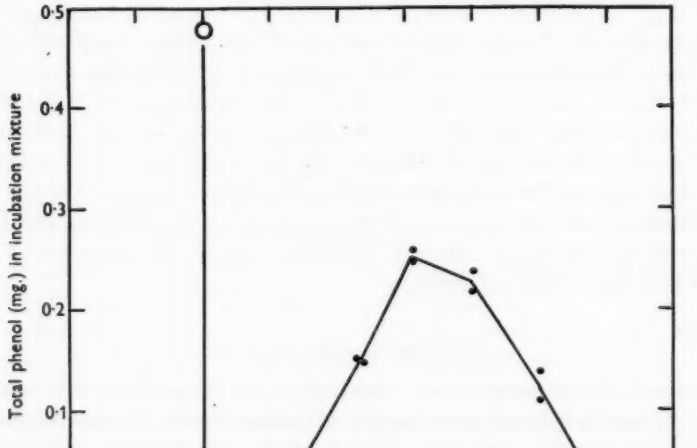
Dry heat, 110° for 60 min., and wet heat at 90° for 2 min., completely inhibited the reaction. Incubation in 0·1 M-NaF completely inhibited the reaction in the acid substrate but had little effect at pH 9·0. The addition of KCN (0·1 %) to the substrates reduced the distribution of the alkaline enzyme to small patches at the base of the cuticular channels but did not alter the distribution of the acid enzyme. This susceptibility of the reactions to heat and inhibitors confirms the fact that the results obtained were the products of enzyme activity. In addition, the dissimilar results produced by using different inhibitors suggest the presence of distinct enzymes with individual pH optima.

(*b*) *Biochemical studies*

Cysticercus. Experiments were undertaken to investigate the relationship between pH and the phosphatase activity of tissue extracts. The results of a typical experiment, which are expressed in Text-fig. 1, show that activity occurred over a wide range of pH but with the maximum in the region of pH 5·0. On either side of this point a rapid loss of activity took place with a levelling out on the alkaline side beginning at pH 6·0. In addition, a very small increase in activity occurred in the region of pH 8·0–9·0. This secondary peak appeared in all experiments, suggesting the presence of slight activity at an alkaline pH. The effect of M/100-NaF on the entire pH range of activity was tested and the results, which are given in Text-fig. 2, show that the activity at pH 5·0 has been almost completely

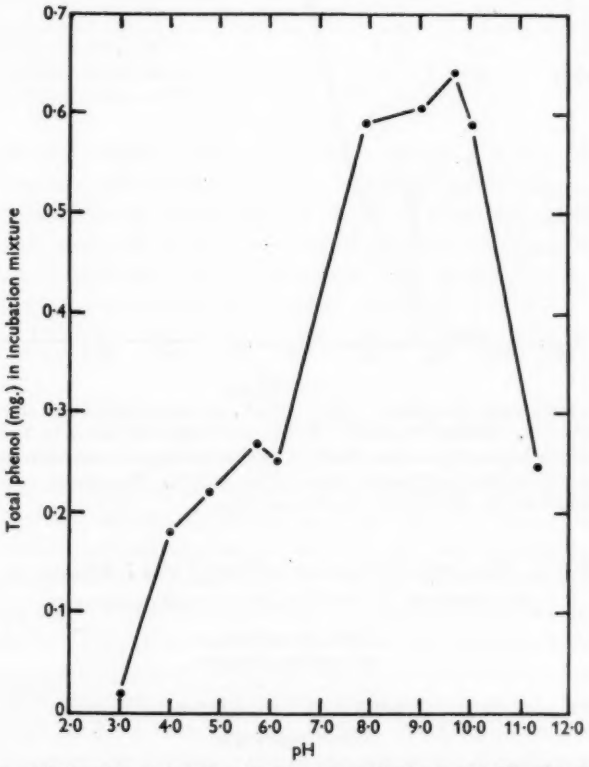


Text-fig. 1. The relationship between phosphatase activity and pH, of extracts of *C. pisiformis*. Extract used consisted of 0·087 g. wet weight (46 cysticerci, 91 days old) in 30 ml. Incubation time 17 hr. at 37°. Denatured controls contained 0·005 mg. of phenol at end of incubation period.



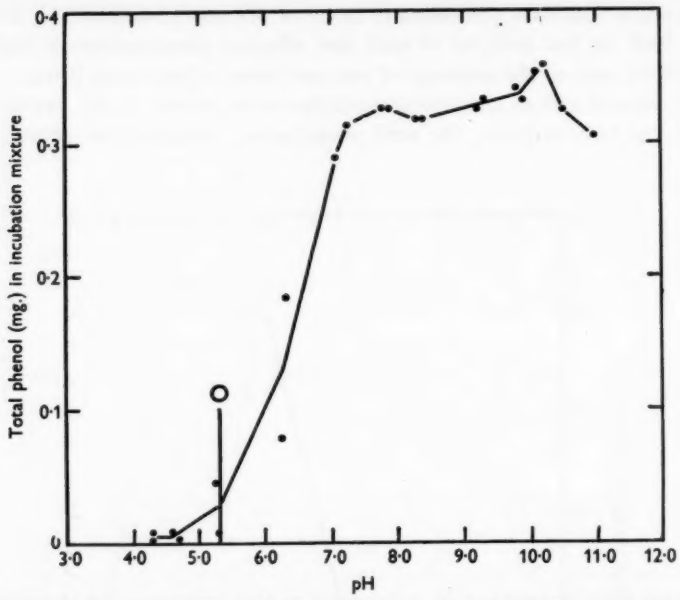
Text-fig. 2. The influence, at different pH's, of M/100-NaF on the phosphatase activity of extracts of *C. pisiformis*. Extract used contained 0·14 g. (74 cysticerci, 116 days old) in 25 ml. O = control tube at pH 5·0 which did not contain NaF in the substrate. All other tubes contained NaF to a final concentration of M/100 in the incubation mixture. Incubation time 19 hr. 30 min. at 37°. Denatured controls contained 0·005 mg. of phenol at the end of the incubation period.

inhibited while that over the alkaline range of pH has persisted. The differential effect of NaF on the activity of acid and alkaline phosphatases is well known (Roche, 1950), and on the evidence of this and other experiments it was concluded that both an acid and an alkaline phosphatase were present in the extracts of this stage. Of the two enzymes, the acid phosphatase exhibited the greater relative activity.



Text-fig. 3. The relationship between phosphatase activity and pH, of extracts of adult *T. pisiformis*. Extract consisted of 0·1 g. wet weight of worm in 30 ml. Incubation time 18 hr. 15 min. at 37°. Denatured controls contained 0·005 mg. of phenol at the end of the incubation period.

Adult tapeworms. Experiments, similar to those described above, were performed on extracts of adult tissue. The activity in relation to pH is given in Text-fig. 3 and the effect of NaF in Text-fig. 4. Again the data indicate the presence of distinct acid and alkaline phosphatases. However, in contrast to the cysticercus, major activity in the adult tapeworm is due to the alkaline phosphatase. Further differentiation was attempted by the use of other inhibitors and activators. These results are given in Table 1. The inhibitors potassium cyanide, potassium oxalate and sodium fluoride exhibited a varying influence depending on the pH at which the experiments were conducted. These figures, plus the evidence given by the



Text-fig. 4. The influence, at different pH's of NaF, on the phosphatase activity of extracts of adult *T. pisiformis*. Extract contained 2·07 g. wet weight of worm in 35 ml. O = control tubes at pH 5·3 which did not contain NaF. All other incubation mixtures contained NaF to a concentration of M/100. Incubation time 18 hr. at 37°. Denatured controls contained 0·005 mg. of phenol at the end of the incubation period.

Table 1. *The action of various activators and inhibitors on the phosphatases of adult tapeworm and cysticercus*

Test	Total phenol (mg.) in reaction mixture: pH 5·0	pH 9·0	Incubation time at 37°
	Adult tapeworm		
Denatured extract	0·01	0·01	23 hr. 20 min.
Normal extract	0·22	0·68	
M/100-NaF	0·06	0·68	
M/100-$MgSO_4$	0·07	0·62	
Denatured extract	0·01	0·01	24 hr. 45 min.
Normal extract	0·20	0·67	
M/100-KCN	0·25	0·64	
M/100-K oxalate	0·14	0·64	
	Cysticercus		
Denatured extract	0·005	0·005	25 hr. 20 min.
Normal extract	0·205	0·115	
M/100-NaF	0·025	0·11	
M/150-$MgSO_4$	0·180	0·11	
Denatured extract	0·003	0·003	17 hr. 30 min.
Normal extract	0·024	0·012	
M/50-$MgSO_4$	0·004	0·008	

Table 2. *Characteristics of the phosphatases of adult tapeworm and cysticercus*

	Adult		Cysticercus	
Test	Acid	Alkaline	Acid	Alkaline
pH optimum	5·0–6·0	7·5–10·0	5·0	8·0–9·0
Activation by Mg^{2+}	−	−	−	−
Inhibition by NaF	+	−	+	−
Inhibition by KCN	−	+	−	+
Histochem. demonstration by method of Glick	+	+	−	−

graphs, confirm that activity over a wide range of pH is due to distinct sets of enzymes and not to a single enzyme with an extensive pH tolerance. The characteristics of the enzymes are summarized in Table 2. It is interesting to note the lack of activation of the alkaline enzyme by magnesium sulphate. This point was not investigated in detail, but other experiments gave similar results. Whether the lack of activation is real or apparent is not as yet clear.

DISCUSSION

Both biochemical and histochemical studies have indicated the presence of acid and alkaline phosphatases in the adult tapeworms. Although biochemical tests demonstrated the activity of both acid and alkaline phosphatases in the cysticercus, histochemical studies consistently gave a negative reaction. This discrepancy may be due to an initially low enzyme concentration which did not survive fixation and subsequent procedures. It has been shown (Danielli, 1946) that up to 75 % of the enzyme activity may be lost in the process of fixation, embedding and demonstration of activity. Thus it is important to realize that a negative histochemical result does not necessarily imply a complete absence of enzyme and confirmation by more sensitive methods should be undertaken wherever possible.

These experiments show that the change from cysticercus to adult is accompanied by a change in the nature of the predominant phosphatase present in the tissues. This change-over is not so absolute as postulated by Pennoit-De Cooman & Van Grembergen (1947), who found a single enzyme (acid-optimum pH 4·5) in the cysticercus and one (alkaline-optimum pH 8·0) in the adult stage of *T. pisiformis*. A similar change in phosphatase activity during the development of an organism has been noted by Mazia, Blumenthal & Benson (1948) in the eggs of *Arbacia* and by Yao (1950) during the development of *Drosophila*. In both these cases the predominant enzyme changed from acid to alkaline phosphatase. The resemblance of these results to those presented in this paper is striking but the physiological significance of such changes is difficult to perceive at present.

Histochemical studies on the adult tapeworm have indicated that the phosphatases occur mainly in the cuticle. It is interesting to note that a similar cuticular location for alkaline phosphatase has been demonstrated in the Acanthocephala (Bullock, 1948) and in the sporocyst of a strigeid cercaria (Erasmus,

unpublished observation). In common with *Taenia pisiformis* these animals lack mouth and anus as well as an alimentary canal. Thus in all three the metabolites, obtained from an environment rich in the products of digestion, must enter through a cuticle which exhibits alkaline phosphatase activity. A similar association may be suggested for the alkaline phosphatase activity exhibited by the membrane surrounding the ovary and vitelline gland. The lobules of these organs are enclosed by parenchymal cells rich in glycogen (see Pl. IX, fig. 5), and in the absence of a vascular system the nutriment needed to maintain the high activity of these organs must be provided by the surrounding cells. Thus these associations tend to support the emphasis placed by Danielli (1952) on the relationship between alkaline phosphatase location and active transport across cell membranes. However, tests have failed to produce conclusive evidence that similar phosphatase activity occurs in the testis lobules of *T. pisiformis*.

The location of phosphatases, as described earlier, implies the active production of free phosphate at those sites. It is therefore suggested that this is linked to the passage of glucose and possibly other substances through the cuticle and internal membranes. The apparent absence of phosphatase activity, which may reflect a low enzyme concentration, from the scolex suggests that this region may not be so actively concerned with the passage of materials through the cuticle as are other regions of the worm. The location of phosphatase activity in the subcuticular cells and the fact that the most well-defined histochemical reaction is given in the region of active, mature proglottides may lend some significant support to this association between phosphatase location and active passage of materials through a membrane. If this is so, the demonstration by Read (1951) of a phosphorylase in the extracts of *Hymenolepis diminuta* indicates that the mechanism may be one of phosphorylation with a secondary role played by the phosphatases as suggested for glucose absorption in the renal tubules and intestine of mammals (Moog, 1946).

SUMMARY

1. The phosphatases present in the adult and cysticercus stages of *Taenia pisiformis* have been investigated using histochemical and biochemical methods.

2. Histochemical tests failed to demonstrate the sites of enzyme activity in the cysticercus.

3. In the adult, the acid phosphatase is confined to the cuticle. Alkaline phosphatase occurs in the cuticle, subcuticular cells and the membranes bounding the ovary and vitelline tubules.

4. The histochemical distribution is uneven along the length of the worm, both acid and alkaline phosphatase being predominant in the region of 'mature' proglottides. The scolex was negative to both tests.

5. Biochemical tests have demonstrated distinct acid and alkaline phosphatases in the cysticercus and adult stages. In the cysticercus the acid enzyme is predominant and in the adult it is the alkaline, implying a change in relative magnitude during the completion of the life cycle.

6. pH-activity curves have been obtained for the enzymes of both stages.

7. The results are discussed in relation to recent findings in the field of cestode enzymology, and it is suggested that these phosphatases may be associated with active transport of materials across the cuticle and ovarian and vitelline membranes.

I wish to acknowledge the financial aid of the Agricultural Research Council, as part of this work was carried out during the tenure of a research studentship. I am also grateful to Prof. J. Brough for his valuable criticisms and advice, and to Prof. J. M. Petersen for providing generous facilities in the Institute of Physiology.

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EXPLANATION OF PLATE IX

Fig. 1. Acid phosphatase reaction in the cuticle of adult *T. pisiformis*. Horizontal section of mature proglottis, incubation time 5 hr. at 37°. Section unstained. × 700.

Fig. 2. Acid phosphatase reaction in the cuticle of adult *T. pisiformis*. Horizontal section of early mature proglottis, incubation time 3 hr. at 37°. Section unstained. × 730.

Fig. 3. Alkaline phosphatase reaction in the cuticle and subcuticular cells of adult *T. pisiformis*. The oval calcareous bodies also gave a reaction in the control mixture. Horizontal section of mature proglottis, incubation time 1 hr. at 37°. Section unstained. × 216.

Fig. 4. Alkaline phosphatase reaction in the cells and tunica of the ovary in a horizontal section of mature proglottis of adult *T. pisiformis*. Incubation time 1 hr. at 37°. Section unstained. × 428.

Fig. 5. The distribution of glycogen (black) in a horizontal section of mature proglottis of adult *T. pisiformis*. The section is unstained but for the demonstration of glycogen. Silver technique of Gomori. × 200.

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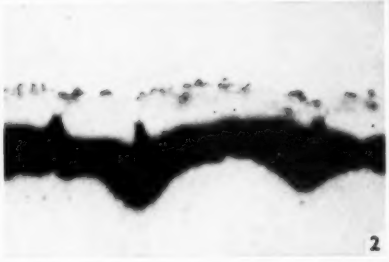

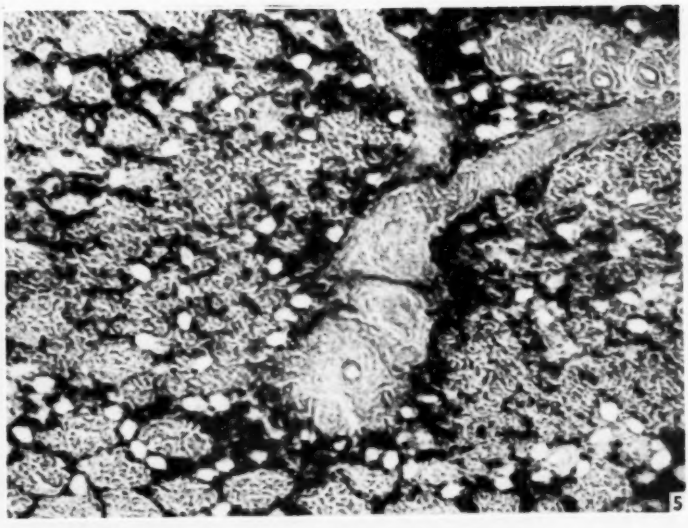

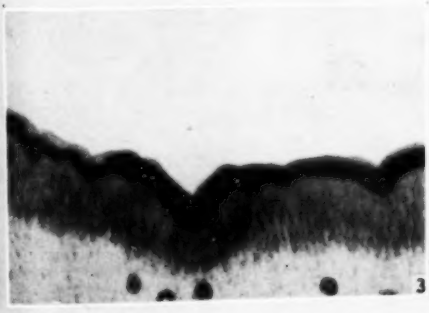

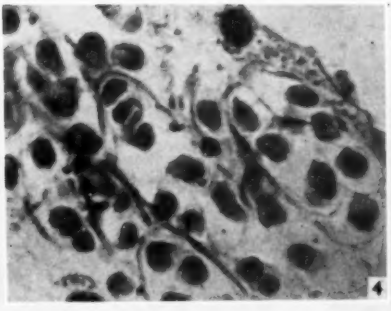

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STUDIES ON PHOSPHATASE SYSTEMS OF CESTODES

II. STUDIES ON *CYSTICERCUS TENUICOLLIS* AND *MONIEZIA EXPANSA* (ADULT)

By DAVID A. ERASMUS

Department of Zoology, University College, Cardiff

(With Plate X and 4 Figures in the Text)

INTRODUCTION

Biochemical studies (Erasmus, 1957) have demonstrated acid and alkaline phosphatase activity in extracts of the cysticercus and adult of *Taenia pisiformis*. During the establishment of the cysticercus in the final host, and its development into an adult tapeworm, a relative change in activity occurs. In the adult tapeworm, alkaline phosphatase activity became predominant, in contrast to the major activity of the cysticercus, which was exhibited by acid phosphatase. Histochemical studies showed that, although most of the acid and alkaline phosphatase activity occurred in the cuticle, some was associated with the reproductive system. This composite study has been extended to include *Moniezia expansa* and *Cysticercus tenuicollis*, in order to confirm previous results and to produce a more comparative picture of the distribution of phosphatases in cestodes. In addition, the abundant supply of material facilitated more detailed investigations into the activity of extracts of adult worm.

MATERIAL

Fresh material was obtained from sheep at Roath Abattoir, Cardiff, and transported to the laboratory in warm (37° C.) mammalian saline saturated with toluene. Each cysticercus was removed from its enclosing membrane before it was included in the extract.

METHODS

(*a*) *Biochemical*

The biochemical procedures employed were similar, with the following exceptions, to those previously described (Erasmus, 1957). The abundant supply of material enabled all extracts to be made up to a standard strength of 10% wet wt./vol. Extracts were aqueous and non-buffered. The citrate and borate buffers previously included in the incubation mixtures were replaced by glycine-NaOH (Sorensen) and barbiturate-acetate-HCl (Michaelis) buffers. All solutions were saturated with toluene.

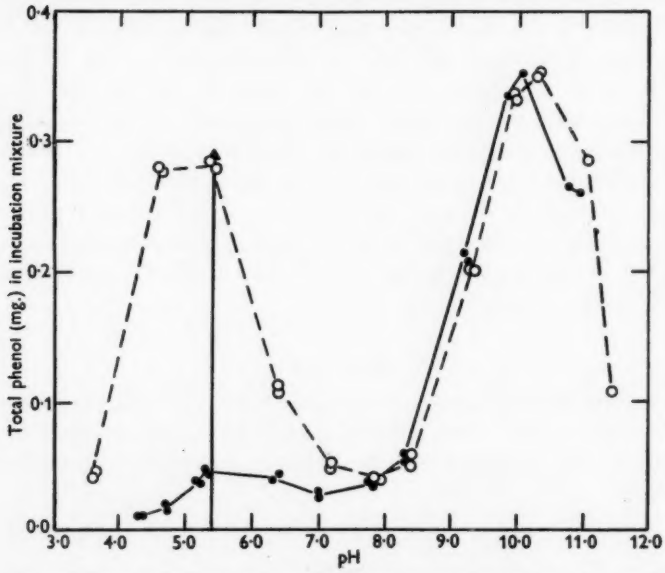
(*b*) *Histochemical*

Two different techniques were employed to demonstrate alkaline phosphatase activity. The majority of the tests were carried out by a modification of the cobalt sulphide technique of Glick. Disodium phenyl phosphate was the substrate and

the pH of the incubation medium was maintained with glycine and barbiturate buffers. The procedure of Glick (1949) was used to demonstrate the sites of activity.

The distribution of alkaline enzyme was confirmed by a second technique employing frozen sections and a coupling azo-dye method (Pearse, 1953). For this, the tissues were fixed in 4% formaldehyde + 1% acetic acid at 4° C. for 1 hr. The material was washed in running water, incubated at 37° C. in 15% gelatine overnight, and then blocked in 30% gelatin. After cooling, the blocks were hardened in 40% formaldehyde for 1 hr., washed in running water and then stored in the refrigerator until needed. Sections were cut at 20, 30, 40 and 60μ in the horizontal plane. The substrate used was sodium α-naphthyl phosphate and the diazotate of 4-benzoyl amino-2:5-dimethoxyaniline was employed for coupling. Incubation was carried out at room temperature for $\frac{1}{2}$–1 hr.

The sites of acid phosphatase activity in acetone-fixed material were demonstrated with the lead sulphide technique of Glick (1949).



Text-fig. 1. Phosphatase activity of extracts of *Cysticercus tenuicollis*. ○---○, Expt. Ct. 2; activity of basic extract; incubation, 16 hr. 27 min. at 37° C. ●—●, Expt. Ct. 5; extract + NaF (M/100). ——▶, extract without NaF, control to Expt. Ct. 5; incubation 16 hr. 12 min. at 37° C.

RESULTS

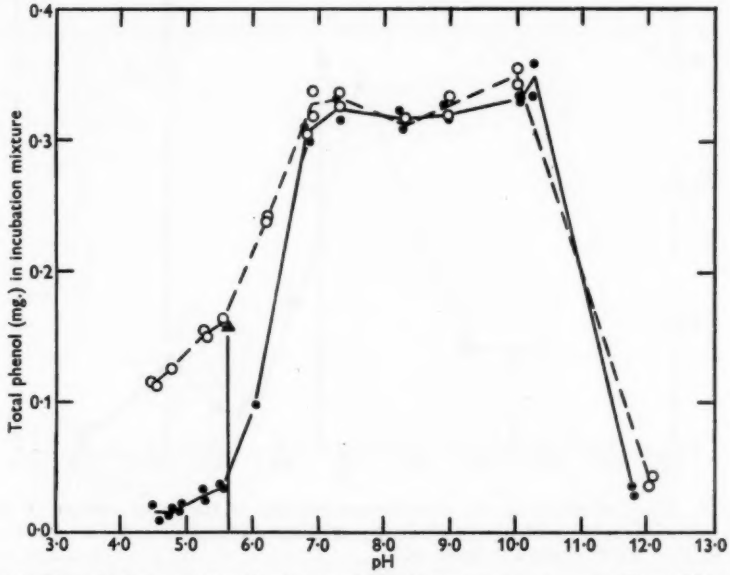
(*a*) *Biochemical studies*

Cysticercus tenuicollis

In the experiments on *Cysticercus tenuicollis* two types of extract were used. One consisted of the standard 10% wet wt. extract of the tissues, and the other of undiluted fluid removed from the bladder.

The relationship between the activity of the tissue extract and pH is expressed in Text-fig. 1. It is apparent that, although activity extended over a wide range

of pH, it was not uniform in its intensity and that two peaks of major activity occurred, one at about pH 5·0 and the other at about pH 10·2. Activity was low over the range pH 7·0–8·5. Sodium fluoride (M/100) produced a considerable depression of the peak at pH 5·0, but had no discernible effect at pH 10·0 (Text-fig. 1). This differentiation by inhibition suggests that one peak was due to alkaline phosphatase, and the other to acid phosphatase activity. In contrast, the bladder fluid exhibited phosphatase activity only over the alkaline range and was represented by a single peak at pH 10·5. It was not possible to determine whether or not this was an inherent activity or represented a 'leakage' from the surrounding tissues.



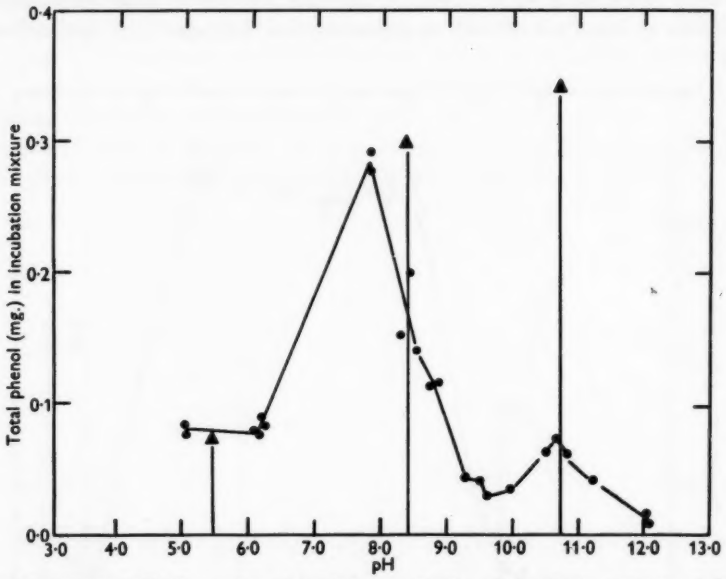
Text-fig. 2. Phosphatase activity of extracts of *Moniezia expansa*. ○---○, Expt. M 3; activity of basic extract; incubation, 17 hr. 6 min. at 37° C. ●—●, Expt. M 4; extract + NaF (M/100); incubation, 16 hr. 56 min. at 37° C. ——▶, extract without NaF, control to Expt. M 4.

Moniezia expansa (*adult*)

Extracts were prepared from entire, mature specimens. The activity of the extract in relation to pH and the effect of NaF upon it are shown in Text-fig. 2. The depression of activity in the region of pH 5·0 suggests the presence of both acid and alkaline enzyme activity. The alkaline enzyme maintained a high activity over a wide range of pH and this is represented in the graph by the presence of two peaks, one between pH 6·5 and 8·5 and the other between pH 10·0 and 11·0.

Further differentiation of the alkaline activity was attempted by using the inhibitor KCN. The results are illustrated by Text-fig. 3. Because of the strong inhibitory action of KCN, it was thought that enzyme activity would be completely suppressed except for some activity in the region of pH 5·0 representing the acid

phosphatase. The results obtained indicated that the inhibition of activity by KCN varied at different pH's. At pH 5·0 little effect was observed; in the region pH 6·5–8·5 enzyme activity was high and differed little from that of the control extract without KCN. Finally, over the range pH 10·0–11·0 marked inhibition occurred. Thus inclusion of KCN in the incubation mixtures differentiated the alkaline phosphatase activity (as previously demarcated by NaF) into two zones differing in their sensitivity to KCN.



Text-fig. 3. Effect of KCN on the phosphatase activity of the basic extract of *Moniezia expansa*; incubation, 17 hr. 37 min. at 37° C. ●—●, Expt. M 7; extract + KCN (M/100). ⟶, extract without KCN, control to Expt. M 7.

The histochemical studies on *Taenia pisiformis* showed that there was an uneven distribution of enzyme along the length of the worm, with the greatest activity in the region of the mature proglottides. Chance observations suggested that the alkaline activity resistant to KCN demonstrated in *Moniezia* was concentrated in the region of mature proglottides. In an attempt to localize this activity, an extract was prepared of a specimen from which a portion of the strobila containing the mature proglottides had been removed. The normal activity of this extract, the effect of KCN upon it and its relationship to the activity of a complete specimen is illustrated in Text-fig. 4. In addition, the activity of a control series containing denatured extract, buffer, substrate and KCN is included. All the extracts were of the same wet wt./vol. concentration. From these results it can be seen that the activity of mixture A (complete animal + KCN) is much greater over the region pH 5·0–8·0 than that of mixture C (animal minus region of mature proglottides + KCN). This shows that a large proportion of this alkaline activity resistant to KCN is located in the region of the mature proglottides. The removal of this zone

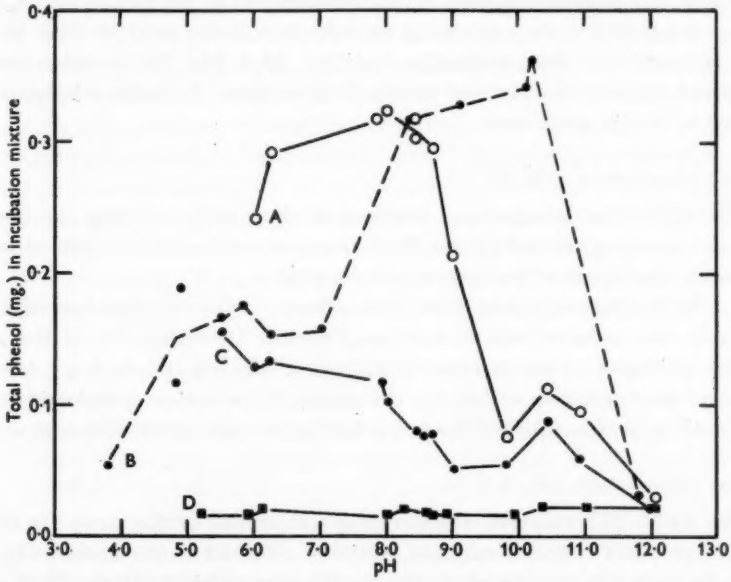
did not seem to affect the activity of the acid phosphatase or the alkaline activity sensitive to KCN to any marked extent (mixture B, animal minus region of mature proglottides, no KCN), although the histochemical tests to be described later showed that these enzymes are present in ample quantities in this region.

The results of these experiments suggest the presence of three types of phosphatase activity in aqueous extracts of adult *Moniezia*.

Type 1. Acid phosphatase, optimum pH 5·0–6·0, inhibited by NaF but not by KCN.

Type 2. Optimum pH 7·0–8·0, resistant to NaF and to KCN. This activity seems to be concentrated in the region of the mature proglottides.

Type 3. Optimum pH 10·0–11·0, not affected by NaF but markedly inhibited by KCN.



Text-fig. 4. The relative effect of KCN (M/100) on the phosphatase activity of extracts of entire and incomplete *Moniezia expansa*. ○—○, mixture A; complete worm + KCN. ●---●, mixture B; worm minus region of mature proglottides, no KCN. ●—●, mixture C; as B + KCN. ■—■, mixture D; denatured extract + KCN. Incubation time of all mixtures, 16 hr. 43 min. at 37° C.

Earlier work on *Taenia pisiformis* showed that the alkaline phosphatase activity was expressed as a double peak with one maximum in the region pH 7·0–8·0 and a second in the range pH 10·0–11·0. This was not commented on at the time, but it is possible that these two peaks may be the product of several types of enzyme activity exhibiting different characteristics as in *Moniezia*.

(*b*) *Histochemical studies*

Cysticercus tenuicollis

Histochemical attempts to demonstrate the sites of phosphatase activity by the sulphide and the diazonium coupling methods were unsuccessful.

Moniezia expansa

An attempt has been made to demonstrate the location of the three enzyme groups revealed by the biochemical studies. For this, three basic incubation mixtures have been employed: (1) acid substrate, pH 5·0; (2*a*) substrate, pH 8·0; (2*b*) substrate, pH 8·0 + KCN; (3*a*) substrate, pH 10·0; (3*b*) substrate, pH 10·0 + KCN.

Acid phosphatase, pH 5·0

All reactions were confined to the cuticle. The exact picture produced within the cuticle depended on the time of incubation, the initial reactions being confined to the walls of the elaborate system of channels present in the cuticle (Pl. X, fig. 1). Increase in incubation time produced an extension of the reaction from the walls of the channels into the surrounding cuticle. After 3 hr. the reaction was very intense and masked all structural details of the cuticle. A similar relationship was recorded in *Taenia pisiformis*.

Alkaline phosphatase, pH 8·0

(i) *No* KCN. The reaction was confined to the cuticle covering the body and lining the interproglottidal glands. The strongest and most well-defined reaction occurred in the region of the mature proglottides.

(ii) + KCN. This inhibitor had little effect on the distribution of enzyme activity in the region of the mature proglottides. On either side of this central zone the resistance of the enzyme to inhibition was less, producing a linear distribution with maximum activity in the region of the mature proglottides. Some of this activity resistant to KCN was located in the walls of the cuticular channels.

Alkaline phosphatase, pH 10·0

(i) *No* KCN. This reaction was the most widespread of the three. In common with the previous types of enzymes, activity occurred in the main body cuticle (Pl. X, fig. 3) and in the cuticle lining the interproglottidal glands (Pl. X, fig. 4). Both the subcuticular cells and the cells of the interproglottidal glands were active. The tunica bounding the lobules of the ovaries, vitellaria and testes (Pl. X, fig. 5) gave a well-defined reaction, and, in its early stages of branching and expanding through the parenchyma, the uterus wall also exhibited strong phosphatase activity. However, at a later stage, when the uterus was fully formed, the enzyme activity had greatly diminished and little reaction occurred (Pl. X, fig. 6). A less well-defined irregular reaction occurred along the length of the ventral, longitudinal excretory vessel.

(ii) + KCN. Activity was restricted to a few scattered patches in the cuticle.

Alkaline phosphatase (*diazonium reaction*)

In addition to the tests based on the cobalt sulphide method, the distribution of alkaline phosphatase was studied by a diazonium-coupling technique (Pearse, 1953). Frozen sections of unfixed, non-embedded material and of fixed, embedded

(gelatin) material were tested. At pH 9·0 (the recommended pH of the reaction mixture) the results obtained were similar to those demonstrated by the cobalt sulphide method.

(c) '*In vitro*' studies with living material

Internal activity

The phosphatase activity of the tunicae surrounding the reproductive system was demonstrated in fresh material in the following manner. Short lengths of fresh, living *Moniezia* were cut obliquely in a dorso-ventral direction, thus providing wedges of material in which the various organs were exposed along the oblique plane. These pieces, after being thoroughly washed in saline, were incubated at room temperature in the diazo-substrate. After periods of 1–2 hr. the pieces were removed and examined under the binocular microscope. In those cases where successful wedges were obtained, the tunicae enclosing the reproductive system showed up as a series of black lines against a reddish brown background, thus demonstrating the presence of activity in unfixed, fresh material.

The enzymes described in the internal organs can hydrolyse substrates present in the body fluids and tissues. The cuticular enzymes, however, might be capable of hydrolysing a substrate present in the contents or secretions of the intestine. This hydrolysis could occur at the external cuticular surface of the worm or within the cuticle after absorption of the substrate. The response of entire, living tapeworms to an external substrate was investigated using the diazo-incubation medium employed in the histochemical tests. This mixture was suitable because any breakdown of the α-naphthyl phosphate resulted in the production of the black azo-dye.

Fresh *Moniezia* was thoroughly washed in saline before being placed in a Petri dish containing the substrate. Incubation was carried out at room temperature (18° C.). Breakdown of the substrate occurred within 2 or 3 min., and the entire surface of the animal was soon covered in a reddish brown film containing black particles of the azo-dye. The rate of decomposition was not uniform but seemed to be more intense along the posterior edge of each proglottis. As the reaction proceeded, the by-products diffused outwards from the edge of the tapeworm. This is illustrated by fig. 7, Pl. X. The alternating light and dark bands of dye in the photograph correspond to the difference in activity along the proglottides, the more rapid breakdown producing the darker bands of dye. Over the period of the experiments, no enzyme activity was observed in tapeworms which had been denatured by immersion in distilled water at 90° C. for 30 min. If a piece of cuticle, which had been dissected off a proglottis removed from a worm after a few minutes' incubation, was examined with the microscope, it could be seen that the walls of the cuticular channels gave a positive reaction (Pl. X, fig. 2). It is important to note that although very different techniques have been employed, the final appearance of the channel system is very similar.

The results of these experiments may be interpreted in two different ways. One interpretation is that the sodium α-naphthyl phosphate penetrates the cuticle and is broken down within this layer. The α-naphthol released by enzymic hydrolysis diffuses outwards and couples with the diazonium salt at the surface of the

proglottis. An alternative suggestion is that the tapeworm secretes into the external environment a mucus which is rich in phosphatases and which would react with the substrate at the body surface producing the result described.

In addition to these experiments with *Moniezia*, fresh, entire specimens of *Cysticercus tenuicollis* were tested using the technique previously described. In contrast to the negative histochemical results, a rapid breakdown of substrate took place, with the formation of the black azo-dye at the surface of the cysticercus. The intensity of the reaction was heavier in the neck portion than in the region of the bladder. Thus, it is probable that some of the alkaline phosphatase activity recorded by the biochemical techniques is localized in the cuticle of the cysticercus.

DISCUSSION

The experiments with *Moniezia expansa* and *Cysticercus tenuicollis* have shown that the tissues contain abundant phosphatases and have confirmed the original findings with *Taenia pisiformis*, in which the major phosphatase activity of extracts of adult tapeworm occurred over an alkaline range of pH. The phosphatase activity of *Cysticercus tenuicollis* differs from that of *Taenia pisiformis* in that the alkaline phosphatase exhibited the same degree of activity as the acid enzyme, with the result that the difference between the activity of the two types of enzyme was not so marked.

The histochemical distribution of enzymes is essentially the same as in *Taenia pisiformis*. The biochemical and histochemical tests have revealed in *Moniezia* a zonation of enzyme activity, similar to that recorded in *Taenia pisiformis*, along the length of the strobila. This is further emphasized in *Moniezia* by the irregular breakdown by living specimens of substrate external to the body. It is probable that this uneven distribution reflects metabolic differences between proglottides at different stages of development. This zonation may be exhibited by substances other than phosphatases and the information obtained by Daugherty (1955), who recorded a qualitative difference in the glycogen content of various regions of *Hymenolepis diminuta*, supports this idea.

The studies with *Moniezia* have also confirmed that the cuticular enzymes originate in the walls of the ramifying cuticular channels and that any increase in incubation time produced an extension of activity into the general cuticle. The experiments with living tapeworms suggest the possibility that extensive breakdown of substrate occurs at the cuticular surface. It is possible that these results may be due to the presence of phosphatases (either directly or by contamination) in the mucoid secretions of the subcuticular cells. During the earlier studies on *Taenia pisiformis* occasional sections showed plugs of phosphatase-active material in transverse sections of the cuticular channels and also a granular phosphatase-active deposit on the cuticular surface (Erasmus, 1957, Pl. IX, fig. 2). So far, similar evidence has not been observed in *Moniezia*.

The experiments described thus confirm the presence of an extensive system of phosphatases in cestodes. The activities of these enzymes result in the release of phosphate ions, and these may be utilized in several ways. It is possible that

release of phosphate at the cuticular surface may be correlated with the phosphorylated passage of substances through the cuticle. This suggestion was discussed in an earlier paper in relation to evidence from other parasitic groups (Erasmus, 1957).

A second probable utilization of the phosphate released is in carbohydrate metabolism. The carbohydrate metabolism of helminths has been an active field of research since the early records of abundant carbohydrate reserves in the form of glycogen by workers such as Wardle (1937) and Salisbury & Anderson (1939). Later, more dynamic studies on the rate of utilization of glycogen and the uptake of glucose *in vitro* by Wardle (1937) and many others (see Smyth, 1947), were severely criticized because non-sterile and abnormal culture conditions were used, although results of *in vivo* experiments (Reid, 1940, 1942) supported the rapid rate recorded. The development of an aseptic technique for culture *in vitro* by Smyth (1955) has done much to eliminate earlier criticisms. Hopkins (1952), using this technique and working with *Schistocephalus solidus* (plerocercoid), showed that glycogen utilization was rapid and also that glucose was removed from the culture medium by the worms under completely aseptic conditions. He also showed that glycogen utilization was not so rapid in the presence of glucose. The intake of phosphate, as shown by *in vivo* studies on *Hymenolepis diminuta* (Read, 1950), is also very rapid and, in addition, a large proportion of the phosphate absorbed is converted into the various organo-phosphate compounds which form the intermediary stages of the Embden-Meyerhof scheme of carbohydrate degradation. Read (1951), using extracts of *Hymenolepis*, detected nearly all the intermediary compounds and several of the enzymes involved in this scheme of breakdown. Although the production of fatty acids has been recorded under aseptic conditions (Smyth, 1949), the mechanism of their formation has not been elucidated.

This recent work demonstrates that the phosphorus metabolism of cestodes must be very well developed, and is obviously intimately linked up with carbohydrate degradation. It is not surprising, therefore, to find that cestode tissues are rich in phosphatases. Care is needed, however, in the interpretation of the histochemical results, which suggest that the majority of the enzymes occur in the outer regions of the tapeworm. Whether or not carbohydrate degradation occurs mainly in these regions, which are rich (except for the cuticle) in glycogen, remains to be proven.

SUMMARY

1. Histochemical and biochemical studies have demonstrated the presence and distribution of phosphatases in *Cysticercus tenuicollis* and adult *Moniezia expansa*.

2. Biochemical tests have differentiated the following types of optimum phosphatase activity: *Cysticercus tenuicollis*, acid phosphatase, pH 5·0 (approx.); alkaline phosphatase, pH 10·0 (approx.); *Moniezia expansa*, acid phosphatase, pH 5·5 (approx.); alkaline phosphatase, pH 7·0–8·0 and pH 10·0–11·0.

3. Attempts to locate the enzymes by normal histochemical methods were unsuccessful in the case of *Cysticercus tenuicollis*. In adult *Moniezia*, acid and alkaline phosphatase (both types) occurred in the cuticle. Alkaline phosphatase

(pH 10·0–11·0) only was present in the tunicae enclosing the ovary, vitelline gland and testes. This enzyme was also the only one present in the interproglottidal glands, in the wall of the developing uterus and in the subcuticular cells.

4. Tests for all types of phosphatase activity showed that cuticular activity in *Moniezia* originated in the walls of an extensive branched system of channels. This system was demonstrable in the cuticle of fixed and unfixed material.

5. Incubation experiments with entire living *Moniezia* showed that substrates external to the body were capable of being broken down. Similar tests with *Cysticercus tenuicollis* showed that at least some of the alkaline phosphatase activity recorded by the biochemical tests was located in the cuticle.

These studies would not have been possible but for the very generous co-operation of Mr J. H. M. Hughes (Veterinary Officer) and Mr R. B. Poole (Meat Inspector), at Roath Abattoir, Cardiff. I also wish to thank Professor J. Brough for the provision of facilities within the department.

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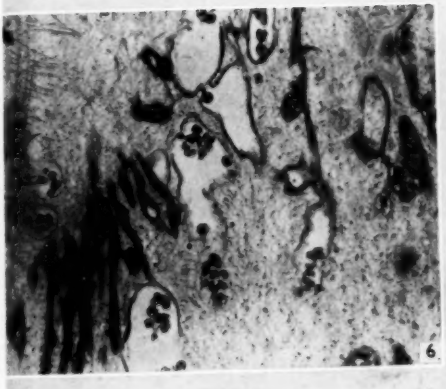
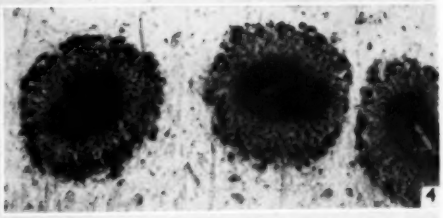
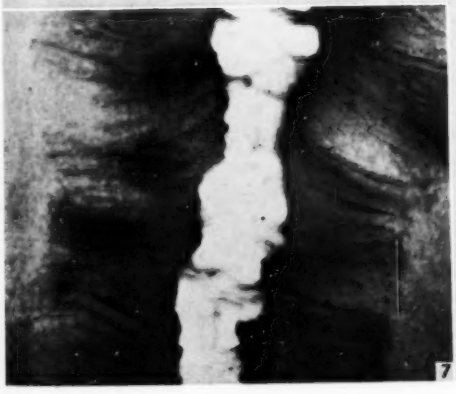
EXPLANATION OF PLATE X

Fig. 1. *Moniezia* (× 441). Acid phosphatase activity in a horizontal section of cuticle parallel to the body surface. Gomori's method, 55 min. at 37° C., pH 5·0.

Fig. 2. *Moniezia* (× 441). Surface view of a portion of cuticle teased from an entire *Moniezia* which had been incubated in alkaline (pH 9·0) diazo-substrate for 2 min. at 20° C. Note the positive phosphatase reaction given by the channel walls.







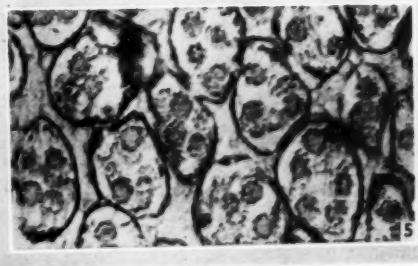


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Fig. 3. *Moniezia* (× 441). Alkaline (pH 10·0) phosphatase activity in a horizontal section of cuticle. Substrate disodium phenyl phosphate; incubation 30 min. at 37° C. Although the most intense reaction occurs in the channels, some activity has extended into the surrounding cuticle.

Fig. 4. *Moniezia* (× 140). Alkaline phosphatase (pH 10·0) in interproglottidal glands. Disodium phenyl phosphate; 30 min. at 37° C.

Fig. 5. *Moniezia* (× 146). Alkaline phosphatase (pH 10·0) in testes lobules.

Fig. 6. *Moniezia* (× 91). Alkaline phosphatase (pH 10·0) in walls of developing uterus. Disodium phenyl phosphate; 40 min. at 37° C. In the expanded regions of the uterus the reaction is not so intense.

Fig. 7. *Moniezia* (× 2). Breakdown of diazo-incubation mixture by living *Moniezia*. The particles of azo-dye produced by enzymic hydrolysis diffuse outwards from the tapeworm body as discrete, alternating light and dark bands. Photograph taken 8 min. after the start of the experiment.

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THE MODE OF EMERGENCE OF THE SPOROPLASM IN MICROSPORIDIA AND ITS RELATION TO THE STRUCTURE OF THE SPORE*

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(With 7 Figures in the Text)

INTRODUCTION

According to the earlier workers on Microsporidia, extrusion of the polar filament from the spore is followed by a creeping out of the sporoplasm in amoeboid fashion through the aperture made by the extruded filament. As early as 1888 Pfeiffer claimed that in hanging-drop preparations of spores of *Nosema bombycis* he saw the sporoplasm emerging through the membrane, but his diagrams are not at all convincing. Later Sasaki (1897) said that in the blood of the silkworm the sporoplasm of *N. bombycis* emerged from one of the poles or from the side of the spore. Paillot (1918) made a similar observation on *Perezia legeri*. Fantham & Porter (1912) described the creeping out of the sporoplasm from the spore of *Nosema apis* through an aperture at the base of the extruded filament, and gave details of the subsequent movements of the amoeboid body over the surface of the intestinal epithelium. Among other workers who have considered that the sporoplasm emerges in this manner are Stempell (1909) and Kudo (1916) for *N. bombycis*, Fantham & Porter (1914) for *N. bombi*, Trappmann (1923) for *N. apis*, Kudo (1925) for *Stempellia magna* and Zwölfer (1926) for *Plistophora blochmanni*.

The function attributed to the filament was to anchor the spore near to the host tissue until it became detached from the membrane, leaving an aperture through which the sporoplasm could emerge. They failed to realize that this view was paradoxical since, first, the long filament would anchor the spore at some distance from the host tissue, leaving the sporoplasm a relatively great distance to travel and, secondly, as Bailey (1954) points out, '. . . the spores would be anchored only to be released when the filament breaks off to allow the planont to emerge'. In the case of *Nosema pulicis* (*N. ctenocephali*) Korke (1916) saw the binucleate sporoplasm at the tip of the polar filament and believed the filament served to conduct the sporoplasm to distant parts of the host tissue.

Since then several workers have noticed a protoplasmic globule at the tip of the extruded filament in the species studied by them. Morgenthaler (1922) described a gush of fluid from the distal end of the extruded filament of *N. apis* but did not

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comment on it. Ohshima (1927) first noted in *N. bombycis* a viscous fluid drop at the tip of the extruded filament which he believed served for adhesion to structures with which it came in contact. Later Ohshima (1937), by staining, demonstrated the sporoplasm at the centre of the fluid drop. He put forward the theory that the polar filament which uncoils during discharge from the spore is a hollow structure and that the fluid sporoplasm emerges through it. The function of the polar filament, he suggested, is to inject the sporoplasm directly into the host tissue. Trager (1937) and Gibbs (1953) have also seen a protoplasmic globule at the tip of the filament in *N. bombycis* and *Gurleya* sp. respectively. Gibbs agrees with Ohshima that the filament is hollow and that the sporoplasm is discharged through it but, in contrast to Ohshima, believes the filament to be everted like the nematocyst of a coelenterate. As Kudo (1944) suggests, a considerable force would have to be applied to the sporoplasm for it to emerge through the narrow tube and that this would probably result in injury to the delicate sporoplasm.

Ohshima (1927) showed that the digestive fluid of the silkworm completely digested the filament in a short time, and Zwölfer (1926) showed in *Plistophora blochmanni* that trypsin completely digests the filaments in 24 hr. These facts suggest that, whatever function is attributed to the polar filament, it must be momentary, and it is most reasonable to accept the recent view that it brings the sporoplasm in some way into close contact with the host tissue, after which it gets digested. It remains to be solved whether the sporoplasm is injected into the tissues, as believed by Ohshima and Gibbs, or whether it is attached to the tip of a solid filament and is merely applied to the tissues (Dissanaike, 1955*a*).

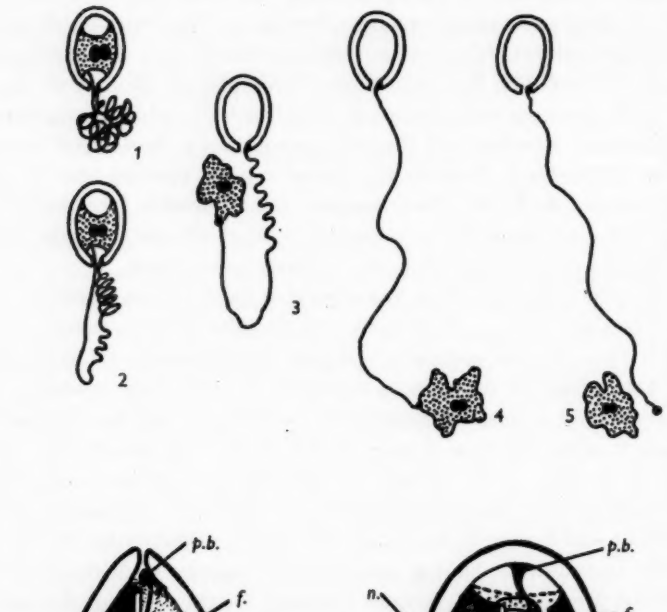
We were fortunate in having available two species of Microsporidia, *Nosema helminthorum* Moniez and *N. locustae* Canning, for the study of this question. The observations on both species were similar and have led us to the belief that the sporoplasm is attached to a solid filament. Furthermore, these observations enable us to draw some useful conclusions on the internal structure of the spores.

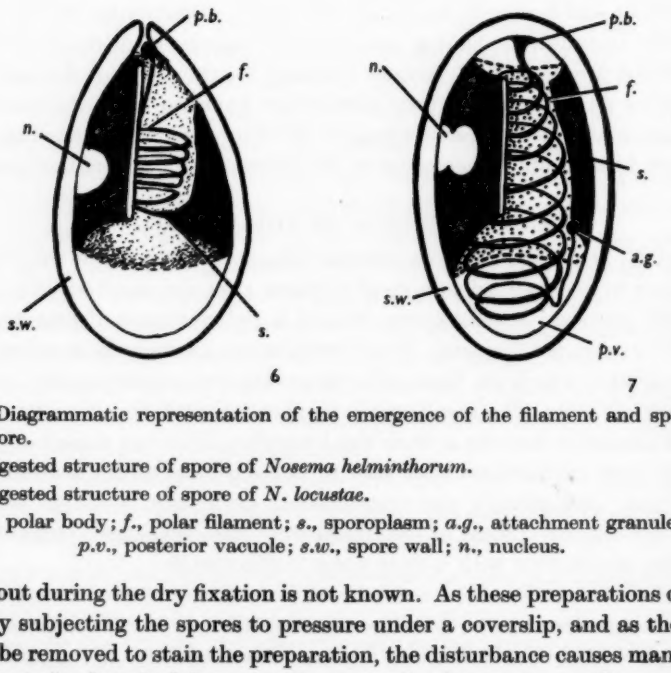
THE EMERGENCE OF THE SPOROPLASM

Spores of *N. helminthorum* from infected *Moniezia expansa* left overnight in the refrigerator were examined in a drop of water that was covered with a coverslip and lightly pressed. Several spores showed a globular mass of protoplasm at the tips of their extruded filaments. The corresponding spores were completely empty. The preparation was dried, fixed, after removing the coverslip gently, and stained with Giemsa's stain. The result was a preparation with over twenty spores with extruded filaments showing at their tips irregular spread-out masses of protoplasm which on close examination were seen to be continuations of the filaments. These masses were undoubtedly the sporoplasms, each with a single nucleus also of irregular outline. Some of the nuclei were dumbell-shaped, while others had an annular appearance with a clear space in the middle.

Spores of *Nosema locustae* from infected *Locusta migratoria* treated in the same way needed greater pressure to induce filament extrusion. In fresh preparations the fluid drops noted in the former case were not observed, but in preparations stained with Giemsa several irregular masses were seen at the tips of the filaments (Fig. 4).

Each protoplasmic mass contained two compact nuclei, usually closely apposed but occasionally widely separated. In both cases the sporoplasms are irregular amoeboid bodies when seen in permanent preparations. Whether in fact they are capable of amoeboid movements or whether their irregular appearance is due to





Figs. 1–5. Diagrammatic representation of the emergence of the filament and sporoplasm from the spore.

Fig. 6. Suggested structure of spore of *Nosema helminthorum*.

Fig. 7. Suggested structure of spore of *N. locustae*.

p.b., polar body; *f.*, polar filament; *s.*, sporoplasm; *a.g.*, attachment granule; *p.v.*, posterior vacuole; *s.w.*, spore wall; *n.*, nucleus.

spreading out during the dry fixation is not known. As these preparations can only be made by subjecting the spores to pressure under a coverslip, and as the coverslip has to be removed to stain the preparation, the disturbance causes many of the sporoplasms to be detached from the filaments. In these cases small granules can

be seen at the tips of the filaments in *Nosema locustae*, which no doubt serve as points of attachment for the sporoplasms. Similar observations were made on a few spores of *N. helminthorum* from an experimentally infected *Hymenolepis nana* of a mouse and also in *Nosema buckleyi* Dissanaike (1955*b*) from a pupa of *Tribolium* castaneum. In the latter case a globular mass of protoplasm was seen at the tip of the extruded filament, which though fully extruded appeared short since it was closely coiled.

Observations on filament extrusion of many spores lead us to believe that the tip is the last part to come out of the spore. In cases where the filament is only partly extruded it can be seen as a tightly coiled mass just outside the spore membrane (Fig. 1). At a later stage the filament begins to uncoil, and it can be seen that the distal end is coiled back on the rest and that the tip has not yet emerged (Fig. 2). Spores at this stage, stained with dilute Toluidine blue, show at the anterior end blue masses which are the sporoplasms. When the filament finally straightens, the tip is pulled out of the spore, together with the sporoplasm (Figs. 3–5). At the point where the filament leaves the spore a basal thickening can be seen, particularly in *Nosema locustae*, as a deep red spot stained with Giemsa.

When filament extrusion is brought about by artificial methods, in this case by mechanical pressure, in many cases it is so violent that the sporoplasm gets detached from the tip of the extruded filament and is left behind within the spore or at its opening. It is evident that this led previous workers to the conclusion that the sporoplasm creeps out of the spore after filament extrusion, and also explains the infrequency with which the sporoplasms have been observed at the tips of the filaments. From these observations we conclude that the sporoplasm is attached to the tip of the filament, or is a continuation of the filament, which is not hollow. When the filament is extruded, it does so by a simple uncoiling and the sporoplasm gets dragged out of the spore as the filament uncoils.

If, as Gibbs has suggested, the hollow filament is everted and the sporoplasm emerges through the tubular filament, it would be impossible to explain cases which we have observed, where the sporoplasm has emerged from the spore in spite of the fact that the filament remains tightly coiled. Gibbs himself states that it is necessary for the filament to be completely everted before the sporoplasm can emerge. The other possibility, suggested by Ohshima, that the sporoplasm emerges through a hollow filament but that the filament merely uncoils and is not everted, is also rendered improbable by the fact that in some cases it is possible to see the sporoplasms at the openings of the spores. This, as we have explained, is consistent with the permanent attachment of the sporoplasm to the tip of the filament, but it is unlikely that the sporoplasm would be ejected from a tubular filament whilst the tip of the filament remained within the spore, particularly as the suggested function was to inject the sporoplasm directly into the host cells. As further evidence of the solid nature of the filament we quote the work of Krieg (1955) who, by electron microscope studies on the filament of *Plistophora melolonthae*, showed that it is a solid structure estimated at 0·1 μ thick.

THE INTERNAL STRUCTURE OF THE SPORE

Fresh spores of Microsporidia belonging to the Nosematidae are highly refractile and apart from the posteriorly placed vacuole, visible in some, show no other details of structure even under the phase-contrast microscope. Ideas of the internal structure of these spores have, therefore, been based on the results of fixation and staining. This has led to a great deal of difference in interpretation by different authors.

It was Thélohan (1894, 1895) who, upon treating the spores at 36° C. with pure nitric acid or after diluting it with one third of the volume of water, demonstrated what he believed to be the polar capsule in the microsporidian spore. He regarded it as normally invisible owing to a surrounding coat of protoplasm. In our experience, following this treatment, a deep shadow appears in the spore as a flask-shaped area two-thirds the length and one-half the width (Canning, 1953). This, however, is of a purely temporary nature and disappears after a few minutes. Since then numerous workers have either confirmed or denied the presence of the polar capsule and a review of the literature shows that the views on the structure of the spores fall into two main categories. There are those who believe the spore to be pyriform or ovoidal with a polar capsule occupying the anterior half or two-thirds of the intrasporal space, with the sporoplasm at the posterior pole, e.g. Kudo (1920*b*) for *Nosema apis*, Léger & Hesse (1916) for *Plistophora macrospora*, and Kudo (1920*a*) for *Thelohania magna* (=*Stempellia magna*). On the other hand, there are those who believe the spore to be ovoidal or ellipsoidal with the sporoplasm organized as a girdle-like ring located at the middle of the spore and surrounding the spirally coiled filament, which may or may not be within a polar capsule. For instance, Stempell (1904, 1909) for *Glugea anomala* and *Nosema bombycis*, Mercier (1908) for *Thelohania giardi*, Fantham & Porter (1912, 1914) for *Nosema apis* and *N. bombi*, all considered that a polar capsule was present and that there were several nuclei in the spore, some of which were associated with the formation of the polar capsule and spore membrane. Schuberg (1910), for *Plistophora longifilis* and Zwölfer (1926) for *P. blochmanni*, both believed the polar capsule to be absent and that there was but a single nucleus in the spore, being that of the sporoplasm. Schuberg suggested that the nuclei seen by other workers were nothing else but volutin or metachromatic granules.

Our observations on the spores that have extruded their filaments lead us to suggest an arrangement of the internal structure of the spore in which a polar capsule does not exist. When fresh spores are induced to extrude their filaments and sporoplasms, they become completely empty. Examination of these spores, both fresh and stained, has failed to reveal any trace of the polar capsule. If we assume that a polar capsule exists, the sporoplasm must either be situated with the filament within the polar capsule, or as a compact body or a girdle outside the polar capsule. If the sporoplasm is situated outside the polar capsule, then it would be imprisoned in a compartment shut off by the spore wall on one side and the polar capsule on the other. The polar filament present in the polar capsule could not be in contact with the sporoplasm and thus could not serve to drag out the sporoplasm as has been shown to take place.

All workers on Microsporidia agree that the sporoplasm does not lie within the polar capsule. In our experience there is no doubt that the sporoplasm lies as a girdle close to the spore membrane with the coiled filament occupying the remaining intrasporal space. In this case the polar capsule cannot exist, and we conclude that the flask-shaped body visible after addition of nitric acid is an artifact due to the particularly drastic treatment and probably produced by the contraction of the filament and sporoplasm within the spore. These are soon dissolved, thus accounting for the purely temporary appearance of the dark area. It would appear, then, that the microsporidian spore has a comparatively simple structure consisting of a binucleate or uninucleate sporoplasm and a polar filament.

In *Nosema helminthorum* the filament is spirally coiled round the centre of the longitudinal axis of the spore and is continuous at the tip with the sporoplasm which surrounds it in a girdle-like fashion (Fig. 6). At the point where the filament is attached to the spore membrane it is thickened as a small granule which has been called the 'Polkörper' by Zwölfer (1926). The sporoplasm may be uni- or binucleate when present within the spore, but is always uninucleate after emergence. This implies an autogamous fusion of nuclei at some stage in the development of the spore. In young spores a large metachromatic granule is present in the posterior vacuole. This is apparently used up during development of the spore and is rarely present in mature spores. In *Nosema locustae* the filament is again spirally coiled within the girdle, but is believed also to occupy the posterior vacuole (Fig. 7). The basal thickening (polar body) at the point where the filament is attached to the spore membrane is distinct, and at the distal tip of the filament there is again a small thickening which serves as a point of attachment for the filament to the sporoplasm. The sporoplasm is binucleate throughout the whole of the spore life and after its release from the spore. As the stages of the parasite between the liberation of the sporoplasm and schizogony in the fat body have not been traced, the occurrence of autogamy has not been demonstrated. Metachromatic bodies are not present at any stage of spore development in *N. locustae*.

Finally, mention must be made of the results of an experiment to determine the chemical nature of the spore membrane. The Campbell (1929) modification of the van Wisselingh test for chitin was carried out on the spores of the two species of Microsporidia. A characteristic rose-red or purple coloration was obtained in both lots of spores and in a piece of insect cuticle treated similarly as a control. There was no doubt that the spore wall was composed of chitin. The spores of *N. helminthorum*, which are normally pyriform, appeared rounder after the test. The anterior end of the spore was truncated, and it seems likely that at this end there is a non-chitinous part, through which the filament and sporoplasm can emerge after a suitable stimulus. The nature of this stimulus has not been determined.

SUMMARY

1. Studies on two Microsporidia, *Nosema helminthorum* Moniez and *N. locustae* Canning, have shown that the polar filament serves to conduct the sporoplasm out of the spore.

2. The filament is a solid structure and the sporoplasm is either attached to or is a continuation of it. The filament is first extruded as a tightly coiled mass and, as this uncoils, it drags the sporoplasm out of the spore.

3. The sporoplasm, which is either uninucleate or binucleate, takes the form of a girdle of protoplasm, which lies on the inner side of the spore membrane and surrounds the coiled polar filament. There is no polar capsule.

The work was carried out under the supervision of Professor P. C. C. Garnham, Head of the Department of Parasitology of the London School of Hygiene and Tropical Medicine, to whom we would like to express our thanks for his unfailing help and encouragement.

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THE LIFE CYCLE OF THE BRITISH RABBIT TRYPANOSOME, *TRYPANOSOMA NABIASI* RAILLIET, 1895

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(With 9 Figures in the Text)

INTRODUCTION

According to Laveran & Mesnil (1907), a trypanosome in mammals was first described by Gros in 1845 in Russia from the blood of field-mice and moles. Five years later, Chaussat (1850) saw trypanosomes in the blood of *Mus rattus* in France but thought they were young nematodes. Later Lewis (1879) found them in India in *M. decumanus* and *M. rufescens*. He recognized them clearly as flagellated Protozoa. Kent (1881) and Bütschli (1884) referred this flagellate to the genus *Herpetomonas*, calling it *H. lewisi*; its name was changed to *Trypanosoma lewisi* by Laveran & Mesnil (1901). In the past a number of species have been described which resemble morphologically *T. lewisi*, and for the sake of convenience all such trypanosomes have been referred to the *lewisi* group, to which the rabbit trypanosome also belongs.

Jolyet & Nabias (1891) were the first to discover a trypanosome in the blood of a rabbit, and Railliet (1895) called it *T. nabiasi*. Later Nocard & Leclainche (1903) described this trypanosome in the blood of wild rabbits, while Blanchard (1904) referred to it as *T. cuniculi*. Petrie (1904) was the first to record this trypanosome in British rabbits from Hertfordshire. Later, he (Petrie, 1905) stated that tame rabbits were seldom infected, for 230 were examined with negative results, but out of forty wild rabbits four were found to be infected. Bosc (1905) recorded the presence of the same trypanosome in one out of a dozen wild rabbits bought in Montpellier, and Bettencourt & França (1907) found the same parasite in three out of eleven rabbits from Thomar (Estremadura), but in sixty-nine rabbits from other districts of Portugal no trypanosomes could be found. Manca (1906) recorded trypanosomes, similar to those described by Petrie, in the blood of a wild rabbit from Sardinia. Nicolle (unpublished work, quoted by Laveran & Mesnil, 1907) observed trypanosomes in the blood of cachectic rabbits in Constantinople. Ashworth, MacGowan & Ritchie (1908–9) noted the occurrence of this trypanosome in rabbits in Scotland, and it was also recorded by Laveran & Pettit (1910), who noted the presence of trypanosome cysts in rabbit fleas. Brumpt (1913) demonstrated its development in and transmission by the rabbit flea, *Spilopsyllus cuniculi*, without giving any details. Cazalbou (1913) claimed to have discovered from rabbits in France, a trypanosome measuring 80μ in length with a well-developed undulating membrane and a 10–12μ long free flagellum. According to him, the trypanosome was pathogenic to rabbits since they died,

and it was named *Trypanosoma gigas*. Channon & Wright (1927) found this trypanosome (*T. nabiasi*) accidentally in the blood of a hutch rabbit. Kroó (1936), who studied the immunology of this trypanosome, found that the infection lasted from 52 to 218 days, and that the natural infection showed larger numbers of organisms and lasted longer than the experimental infections. Noury (1954) described a spontaneous mixed infection in rabbits with *T. nabiasi* and a spirochaete (*Spirochaeta hispanica* var. *marocana*).

The trypanosome, of which an account is given in this paper, was demonstrated by me (1956) before the Royal Society of Tropical Medicine and Hygiene in November, 1955.

MATERIAL AND METHODS

The work on trypanosomiasis in British rabbits was started in November 1954, when fifty-eight wild rabbits suffering from myxomatosis were examined by blood culture and a few by fresh blood examination, but none was found infected. The collection of rabbits was stopped in winter (December 1954–February 1955), but in March, when the weather conditions became more favourable, an attempt was made to get the strain from Elstree, Hertfordshire, where, 53 years ago, Petrie had observed this trypanosome for the first time in British rabbits. Three rabbits were caught (with the help of ferrets) on 24 March 1955, and one (R 60) was found swarming with trypanosomes, while twelve fleas collected from this rabbit proved to be infected with flagellates. The fleas were crushed in normal saline and inoculated intraperitoneally into rabbit R 500, which became infected and provided the strain for further experiments.

The blood of rabbit R 60 was inoculated into three baby mice, three baby rats, three baby guinea-pigs and one rabbit, but none of them became infected. Twenty-two more rabbits from the same area were examined, but none was found infected. In July 1955, two rabbits were received from the Pest Infestation Control Division of the Ministry of Agriculture, Surrey. One of them was found infected, but its blood did not infect the laboratory rabbits.

In order to obtain the cytological preparations of the developmental stages of the flagellates in the insect and vertebrate hosts, the following techniques were employed. Films were made from the blood obtained from the rabbit's ear, and impression smears were made from the tissues. The films were dried in air, fixed in methyl alcohol for 2–3 min. and stained for 20–45 min. with Revector Giemsa stain (Hopkin and Williams Ltd.) at a concentration of 40 drops of the stain in 20 c.c. of buffered distilled water, pH 7·2–7·4. Some films were kept in the Giemsa stain (20 drops in 20 c.c. of buffered distilled water) overnight at 37° C., which gave very good results.

For sections, the tissues were fixed in Carnoy's or Duboscq–Brasil's fluids. Sections, 5–8 μ thick, were stained with Delafield's or Ehrlich's acid haematoxylin, Maximow's (1924) Azure II eosin and haematoxylin or Giemsa-colophonium (method of Shortt & Cooper, 1948).

HOST-PARASITE RELATIONS

As noted by Davis (1952) 'The life histories of few *lewisi*-like trypanosomes are completely known, and although repeated efforts have been made to find and describe the reproductive stages of these organisms, few have been successful.... The general course of infection is known for *T. nabiasi* of European rabbits, but reproductive stages have not been described.' This work was accordingly directed chiefly to the elucidation of the reproductive stages and also of the course of infection, which has not been followed before.

Course of infection

In order to determine the course of experimental infection in rabbits, twenty clean animals were inoculated intraperitoneally with infected blood or crushed (infected) fleas collected from wild rabbits. The development was followed by daily counts in three rabbits, while five rabbits were sacrificed at various periods of infection (3–6 days) to obtain the tissue stages. Three rabbits proved to be refractory to infection, while three showed a few trypanosomes for about a fortnight after inoculation, but ultimately lost the infection.

It is well known that in certain *lewisi*-like trypanosomes division forms are found in the blood, but these have not been found in the rabbit trypanosome. Therefore, the course of the infection could be followed only by daily counts of the number of trypanosomes in the blood, as shown graphically in Figs. 1, 2 and 3 for the three rabbits.

Rabbit R 512 (about 1 month old) was inoculated on 27 May 1955 with 3·5 c.c. citrated blood from rabbit R 503 containing 2800 trypanosomes per cu.mm. Trypanosomes appeared in the blood of rabbit R 512 21 hr. after inoculation. The number of trypanosomes remained very low for 6 days, after which multiplication started, and they reached a maximum by the tenth day, i.e. 4 days after the commencement of multiplication, after which there was a steady fall in their numbers, when there remained about 1000 parasites per cu.mm. of blood. The infection ended 73 days (8 August 1955) after inoculation, with the disappearance of all trypanosomes from the blood (Fig. 1).

Rabbit R 517 (697 g.) was inoculated on 8 June 1955 with 3·5 c.c. of citrated blood from rabbit R 503, containing 1600 trypanosomes per cu.mm. Trypanosomes appeared in the blood on the fourteenth day after the inoculation and the parasitaemia showed the initiation of multiplication. During the infection, the number of trypanosomes per cu.mm. of blood increased rapidly to 7000 on the seventeenth day, the fourth day after the incubation period was over, after which there was a steady decrease in numbers with slight fluctuations. The rabbit lost its infection 136 days (22 October 1955) after inoculation when all the parasites disappeared from the blood (Fig. 2).

Rabbit R 519 (607 g.) was inoculated on 8 June 1955 with 4 c.c. of citrated blood from rabbit R 501 containing 1600 trypanosomes per cu.mm. of blood. Trypanosomes appeared in the blood 20 hr. after the inoculation, but their number remained low (100 organisms per cu.mm. of blood) for 13 days, but on the four-

teenth day parasitaemia was at its highest with 5000 trypanosomes per cu.mm. of blood. The rabbit was splenectomized the next day, after which the number of parasites fell to 400 organisms per cu.mm. of blood. Seven days after splenectomy there was once again a steady rise, when on 12 July 1955 the highest parasitaemia (3000 trypanosomes per cu.mm. of blood) was recorded. After that, there was a steady decrease until there were about 800 organisms per cu.mm. of blood. The infection terminated 122 days after inoculation with the disappearance of trypanosomes from the blood (Fig. 3).

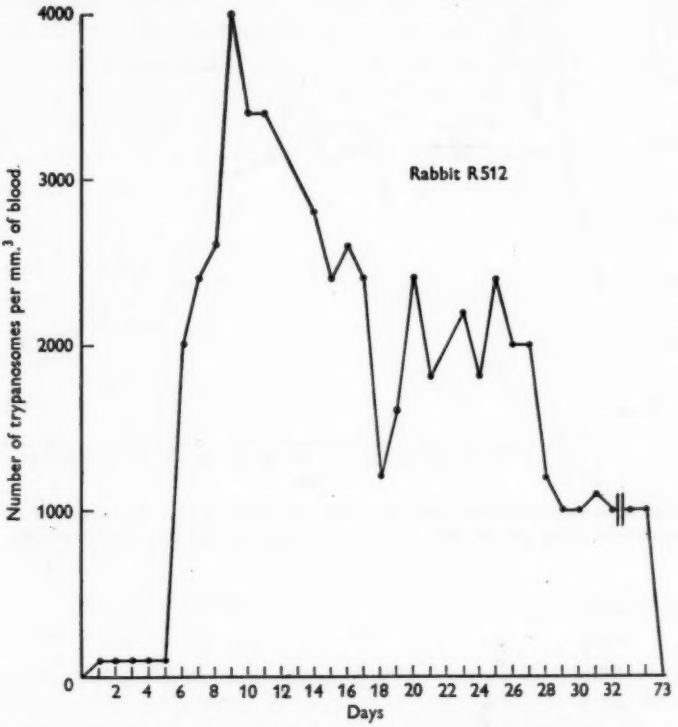

Fig. 1. Course of normal experimental infection in rabbit R512. The number of trypanosomes appearing per cu.mm. of blood throughout the infection is shown.

According to Ashworth *et al.* (1908–9), Channon & Wright (1927) and Kroó (1936), after an incubation period of 7 days, trypanosomes appear in the blood and increase rapidly in number, reaching a maximum on the tenth to twelfth day of the infection. Their number remains high for 3–5 weeks and then falls gradually until the end of the infection. In my experiments, the incubation period varied from 5 to 12 days (though in a few rabbits the trypanosomes appeared in the blood as soon as 20 hr. after inoculation), but the number remained low till the end of the incubation period (5–12 days) when the number increased rapidly, reaching its maximum on the second to fourth day after completion of the incubation period. After this there was a steady decrease (see Figs. 1, 2, 3, 4).

Effect of splenectomy

Taliaferro, Cannon & Goodloe (1931) and Taliaferro & Pavlinova (1936), in their work on *Trypanosoma lewisi* and *T. duttoni*, showed that splenectomy,

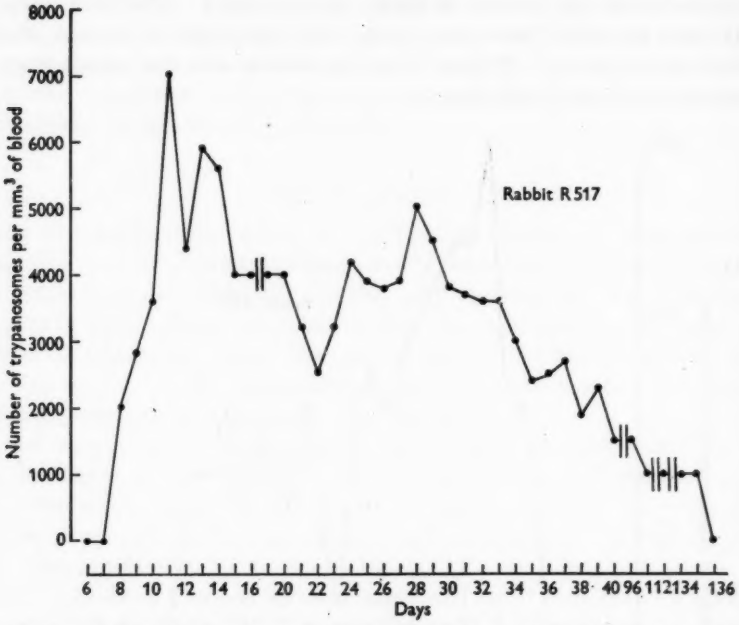

Fig. 2. Course of normal experimental infection in rabbit R 517. The number of trypanosomes appearing per cu.mm. of blood throughout the infection is shown.

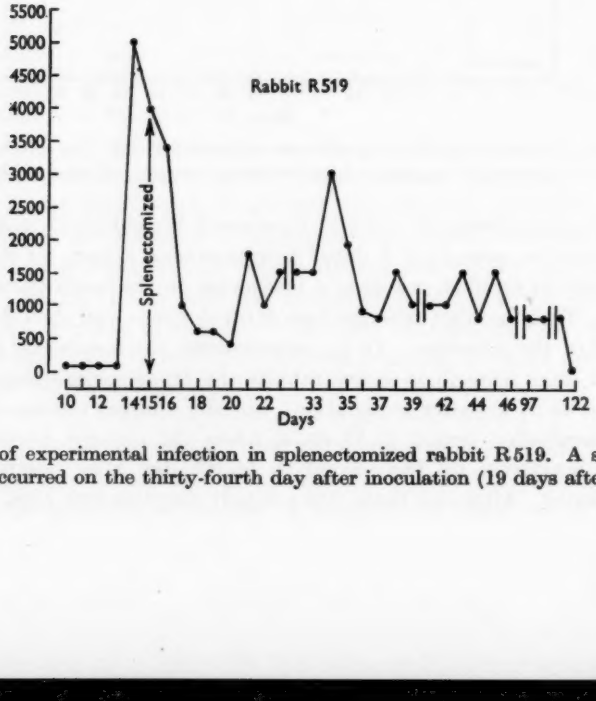

Fig. 3. Course of experimental infection in splenectomized rabbit R 519. A second reproductive cycle occurred on the thirty-fourth day after inoculation (19 days after removal of the spleen).

which reduces the functional level of the reticulo-endothelial system of the rat or mouse, may alter the normal course of infection with these organisms. Splenectomy appears to increase the multiplication period, which continues for a longer period than usual. In order to study the effect of splenectomy on infections with *T. nabiasi* three experiments were performed.

Rabbit R 518 (657 g.) was splenectomized on 6 June 1955, and 2 days later it was inoculated with 3·5 c.c. of citrated blood from rabbit R 501, containing 1600 trypanosomes per cu.mm. After an incubation period of 8 days, during which no trypanosomes appeared in the blood, there was a steady rise in number until on the fourteenth day after inoculation (sixth day after the incubation period) the number of individuals per cu.mm. of blood reached the peak (10,000 trypanosomes), after which there was a steady fall, but the number did not go below 2000 trypanosomes per cu.mm. of blood (Fig. 4). The infection was lost 143 days after inoculation with the disappearance of all the parasites from the blood.

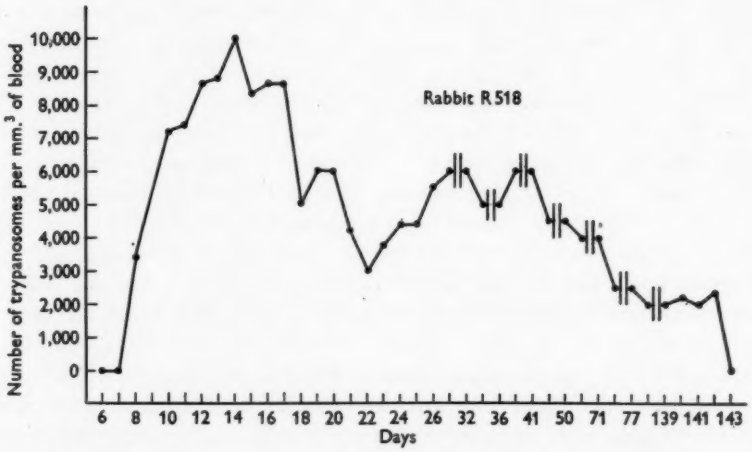

Fig. 4. Course of experimental infection in a splenectomized rabbit R 518. The number of trypanosomes appearing per cu.mm. throughout the infection is shown.

Rabbit R 524 was splenectomized on 23 June 1955, and inoculated on 13 July 1955, i.e. 20 days after the removal of spleen, with 3·5 c.c. of infected blood from rabbit R 514, containing 200 parasites per cu.mm. The rabbit remained uninfected, and 15 days later it was again inoculated with 3 c.c. of blood from rabbit R 501, containing 1000 trypanosomes per cu.mm. The rabbit remained uninfected. Splenectomy performed 20 days earlier did not appear to enhance the infection.

The data regarding splenectomized rabbit R 519 have already been described. This experiment was similar to that of Taliaferro *et al.* (1931), who showed that splenectomy does not markedly influence the rate of development of *T. lewisi* when the operation is performed 41 days after infection.

Effect of other factors

Many earlier workers were unable to transmit the infection from rabbit to rabbit by inoculation of infected blood. Channon & Wright (1927) were able to infect 73% of rabbits by intravenous inoculation only, whereas three rabbits inoculated intraperitoneally remained uninfected. However, in the experiments performed by me, all the inoculations were made intraperitoneally, and if a sufficient number of trypanosomes were inoculated, they were able to produce infection.

The age of rabbits seemed to play some role in the infection, for young rabbits were more susceptible than old ones. One adult rabbit, R 523, which was inoculated intravenously, remained uninfected, while out of two baby rabbits, R 525 and R 526, inoculated similarly, one became infected.

Attempts to infect other animals

It is well known that trypanosomes of the *lewisi* group are typically host-restricted. Thus, according to Laveran & Mesnil (1907), attempts by some workers to infect various rodents (mice, voles, rabbits, hamsters), dogs, goats, horses and monkeys, with *T. lewisi* have failed. Likewise, mice, rats and guinea-pigs proved to be refractory to infection with *T. nabiasi* (Petrie, 1905; Ashworth *et al.* 1908–9; Channon & Wright, 1927). It has been suggested by Wenyon (1926) that most of the *lewisi*-like trypanosomes which have become adapted to particular hosts represent races of *T. lewisi*, and Laveran & Mesnil (1907) emphasized the resemblance of *T. duttoni* to *T. lewisi*. Davis (1952) believed that *T. lewisi* and all *lewisi*-like trypanosomes had evolved from a generalized form which had its origin in an invertebrate and became widely distributed before it became adapted to any one host. In the course of time this form became permanently modified and adapted physiologically to various hosts. After becoming physiologically adapted to a particular host, the ability to pass by direct inoculation from one species of host to another was eventually lost. and certain individual characteristics of development appeared in response to the particular physiology of the host involved.

Roudsky (1910, 1911) was able to infect white mice, guinea-pigs and rabbits with a virulent strain of *T. lewisi*, produced by rapid passage from rat to rat, and Delanoë (1911) was able to infect mice with *T. lewisi*, while Coventry (1929) infected guinea-pigs with this species. In the case of *T. duttoni*, Taliaferro & Pavlinova (1936) showed that the resistance of mice is lowered by splenectomy and India-ink blockade of the reticulo-endothelial system.

In order to ascertain if the same effect could be produced with the rabbit trypanosome, two rats and two guinea-pigs were splenectomized, and 2 days later they were inoculated with the infected blood from rabbits. All the four animals remained uninfected, showing that the removal of the spleen does not lower the resistance in rats and guinea-pigs to *T. nabiasi* infection.

Immunity

Kroó (1936) has pointed out that rabbits after recovering from the infection remain immune to re-infection. In my experiments, three rabbits, R 508, R 511

and R 514, which had lost their infection within 25, 44 and 93 days respectively, were inoculated twice to see if they could be re-infected, but they remained immune to further infection.

Rabinowitch & Kempner (1899) (quoted by Laveran & Mesnil, 1907) have shown that the serum of rats recovered from infection protects these animals against re-infection with *T. lewisi*. In order to ascertain whether there was any cross-immunity between rabbit and rat trypanosomes, the following experiments were made:

(1) Four rats were infected with *T. lewisi*, and on the third day after inoculation trypanosomes appeared in the blood of all of them. Then two rats were inoculated with 0·5 c.c. of immune serum from rabbits; one was inoculated with 0·5 c.c. of immune serum from a rat, and one was kept uninoculated. The third rat acted as a control for the first two and the fourth rat as control for the entire experiment. There was no effect on the rats inoculated with immune rabbit serum, while the third rat lost the infection within a week. Fifty-eight days after the inoculation of immune serum, the first and second rats which were inoculated with rabbit's serum were found to have lost their infection, while the control rat was still infected.

(2) Two rats were inoculated with immune rabbit serum and one with *T. lewisi* immune serum from a rat, while a control one was not inoculated. These four rats were then infected with *T. lewisi* at the same time. The result was very much like the one obtained from the first experiment: out of two rats inoculated with rabbit serum, one lost its infection 49 and the other 73 days after the infection.

These two experiments, though performed on a small number of animals, show that the serum from rabbits infected with *T. nabiasi* has comparatively no protective action against *T. lewisi* infection.

Pathogenicity

According to Jolyet & Nabias (1891) (quoted by Laveran & Mesnil, 1907) 'the health of the animals does not seem to be affected by the presence of this animalcule, even though it be present in the blood in very large numbers...the parasite is to be met with more often in rabbits which are wasted and ill, or in those which have suffered from diarrhoea, than in healthy rabbits'. Petrie (1904) has shown that the trypanosome-containing blood appears to be toxic, for four out of ten rabbits died, without any signs of bacterial infection. Nicolle (quoted by Laveran & Mesnil, 1907) mentioned that the trypanosomes were pathogenic to rabbits, but later workers could not find any rabbits in which the infection ran a pathogenic course.

I have not come across any rabbit in which this trypanosome was pathogenic. It was found to behave like *T. lewisi* in rats. Out of eighty-six wild rabbits, two, R 60 and R 85, which were naturally infected, did not shown any signs of ill health and were also free from myxomatosis, while of more than twenty-five rabbits utilized in the experimental work, no animal died from the infection.

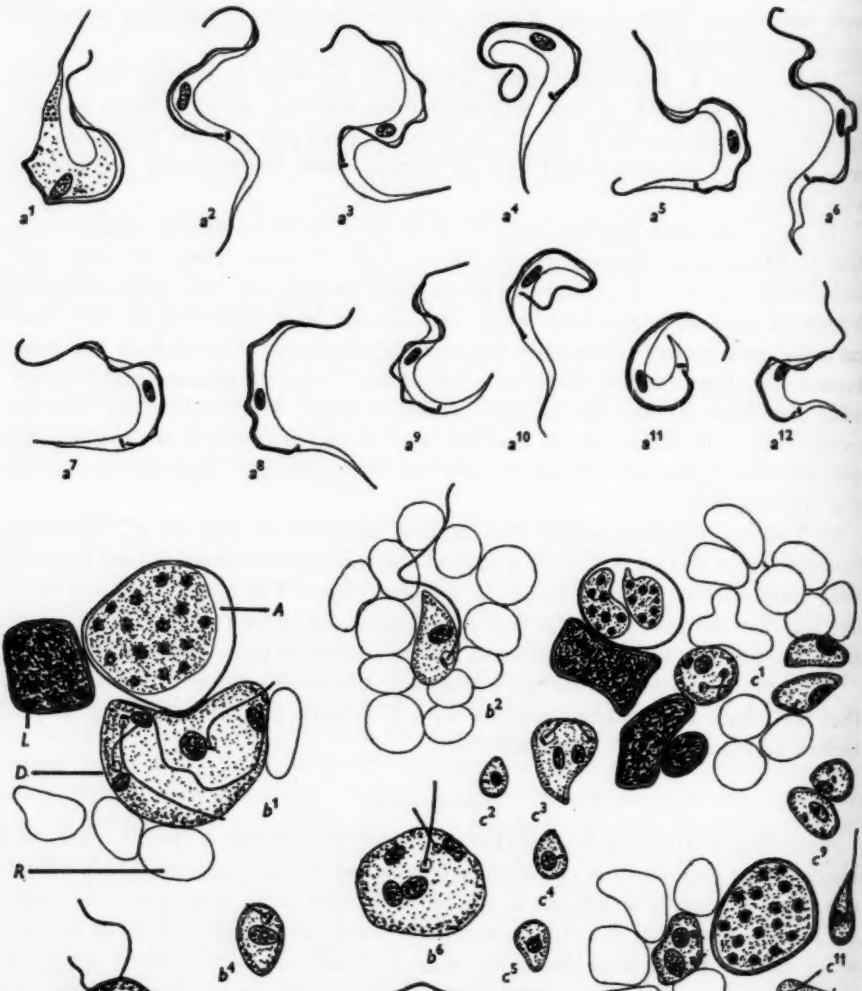

Fig. 5. a^1–a^{12}, trypanosome forms of *T. nabiasi* in the peripheral blood during the multiplication period. b^1–b^7, reproductive forms of *T. nabiasi* in the blood capillaries of the spleen of rabbit. (In b^1, A = endothelial cell; L = lymphatic cell; D = reproductive (division) stage; R = red blood corpuscle). b^8, a developing form in the process of giving rise to a trypanosome. c^1–c^{12}, reproductive forms of *T. nabiasi* in the blood capillaries of the spleen of rabbit.

LIFE CYCLE

Development in the rabbit

Since division forms were absent in the blood of infected rabbits, a number of these animals were examined for the possible presence of reproductive stages in their tissues. These animals comprised two naturally infected wild rabbits, R 60 and R 85, one tame rabbit, R 502, inoculated with naturally infected crushed fleas, and four tame rabbits, R 506, R 507, R 513 and R 519, inoculated with citrated infected blood from artificially infected rabbits. The division stages (Fig. 5, b^1–c^{12}) could be seen only in the spleen of two rabbits, R 502 and R 519. According to Schuberg & Böing (1925) (quoted by Knowles, 1928), the track of invasion of the body in trypanosome infections after intradermal injections is via the lymphatic system, therefore the lymph glands under the forearm and the thighs were examined in two rabbits before the completion of the incubation period, but no division stages could be observed.

Rabbit R 502 was inoculated intraperitoneally on 15 April 1955 with 30–40 crushed fleas which were collected from wild rabbits. Six days after the inoculation and 1 day after the incubation period, the animal was sacrificed, and impression smears were made of liver, lung, kidney, spleen, bone-marrow and brain, which were also fixed for sections. Division forms could be demonstrated from the impression smears of the spleen and could also be seen in sections, where multiple fission forms were seen in the blood capillaries of the spleen (Fig. 5, b^1–b^7). Division forms were not observed in other organs, although adult trypanosomes could be seen in the smears of the liver, lung and kidneys. The blood smear on the same day showed some unusual forms (Fig. 5, a^1–a^{12}) which differed in size from the adult trypanosomes (Figs. 6, *a–t*; 9, *a*). Rabbit R 519 was inoculated on 8 June 1955 with 3·5 c.c. of citrated blood from rabbit R 501. Multiplication started on 22 June 1955, when the number of parasites in the blood was at its peak (5000 per cu.mm.). The rabbit was splenectomized 24 hr. later, and division stages were observed in the impression smears made from the spleen (Fig. 5, c^1–c^{12}). They were confined to the blood capillaries of the spleen. The other five rabbits were autopsied and tissue impression smears were made, but division stages could not be seen in any of them.

The multiplication of *T. nabiasi* in the blood capillaries of the spleen, which has been demonstrated for the first time in rabbits, is of short duration. It is most active from the sixth to the tenth day after infection and is characterized by the marked increase of the number of trypanosomes in the blood, after which it is on the decline and gradually ceases. Multiplication may commence after inoculation of crushed fleas containing metacyclic trypanosomes or of blood forms. The flagellate becomes irregularly rounded, while its kinetoplast and nucleus divide twice in succession, producing a set of four nuclei, kinetoplasts and flagella (Figs. 5, *b, c*; 9, *m–o*). Occasionally division is irregular, resulting in forms with four nuclei and only two kinetoplasts (Figs. 5, b^6; 9, *n*). The cytoplasm remains unchanged until this process of internal division is completed, after which it undergoes fission, giving rise to four short daughter individuals (Figs. 5, b^2; 9, *p*).

These eventually elongate and become transformed into trypanosome forms (Fig. 9, *q*), which emerge from the blood capillaries of the spleen into the peripheral blood. Some curious, unusual type of trypanosomes are found during the multiplication period, varying in length from 25·8 to 38·2μ (Fig. 5, a^1–a^{12}). Some of them are with posterior end prolonged to a great length, so that it almost resembles

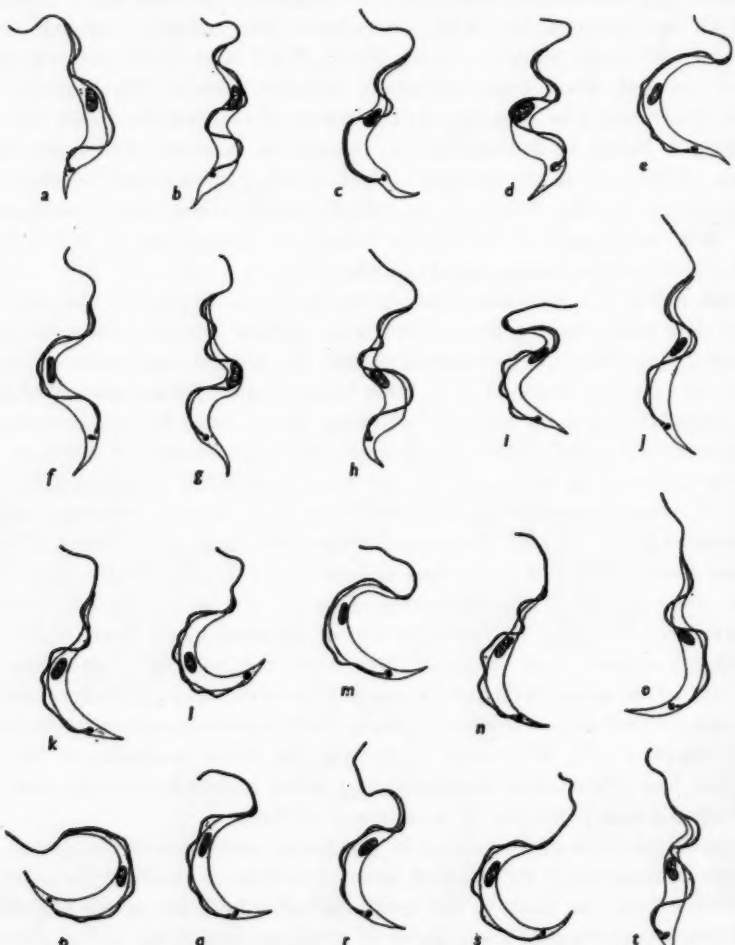

Fig. 6. *a–t*, adult blood forms of *T. nabiasi*.

a second flagellum (Figs. 5, a^3–a^5, a^{10}; 9, *r*). These forms gradually disappear from the blood, and are replaced by the typical 'adult' trypanosomes (Figs. 6, *a–t*; 9, *a*). At no stage of the multiplication are the various dividing forms (leptomonad, leishmanial and crithidial, described by Wenyon (1926) from the

blood of rats infected with *T. lewisi*) found in the blood of rabbits infected with *T. nabiasi*.

The adult trypanosome in fresh blood, on the slide, examined either in film or hanging-drop preparations, appears as a colourless, actively moving body amid the red blood corpuscles which are thrown about by its flagellum. The movements, when compared with those of *T. lewisi*, which is the most motile of all trypanosomes, are less vigorous. In stained preparations the typical adult trypanosome,

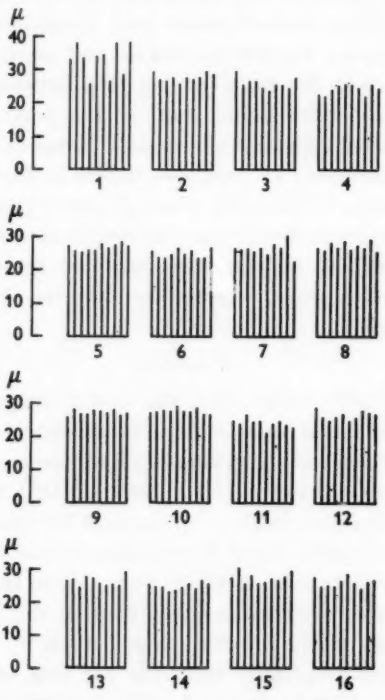

Fig. 7. Lengths of ten trypanosomes taken on each day in the course of infection in rabbits, arranged in order of their lengths in microns. Note greater variation in number 1, and minor variations throughout the course of infection.

which persists in the blood for the longest time after the multiplication phase (Figs. 6, *a–t*; 9, *a*), is very much like the other *lewisi*-like trypanosomes. The organism has a distinctly curved body which is pointed at the posterior end, with a small kinetoplast situated at a distance of about 2–4μ from the posterior extremity. The nucleus occupies a central position in the body, if the length of the free flagellum is excluded. The undulating membrane is not very well developed, and there is a long free flagellum.

Measurements were made of 150 trypanosomes taken from rabbits, starting from the day when the incubation period was over. It was found that on the day when division stages were encountered in the spleen (second day after the incubation period) some unusual forms varying in length from 25·8 to 38·2μ were

present (Fig. 5, a^1–a^{12}), but later on there was little variation and no significant difference in the dimensions (Fig. 7).

In the first description given by Jolyet & Nabias (1891) the length of the rabbit trypanosome was given as 30–36μ with 15μ long free flagellum. Ashworth *et al.* (1908–9) have carefully compared twenty specimens of their strain with those of Petrie's strain, and found that their parasite was smaller, its length range being 27–29·4μ, with an average of 28·4μ, while Petrie's strain varied from 28 to 31μ, averaging 29·0μ; out of twenty specimens of the latter strain only two were over 31μ, while the others were all under 30μ. Channon & Wright (1927) have given an average of 24·17μ for 300 specimens, and according to them the parasites did not vary much in size from the day they appeared in the blood. In my careful measurements, I have found that, at the period when the division is taking place in the blood vessels of the tissues, forms of various sizes appear in the blood, and the coefficient of variation differs in the same way as in other *lewisi*-like trypanosomes. Ultimately, when all the trypanosomes become adult, the coefficient of variation is lower, viz. 150 specimens varied from 21·0 to 30·2μ with an average of 26·72μ; among these only two specimens were above 30μ, while only six were under 23·0μ, whereas the majority of the trypanosomes were between 26 and 28μ long.

Viability

Jolyet & Nabias (1891) found that the rabbit trypanosome survived at a temperature near zero for at least 5 days in an ordinary slide preparation, in which the edge of the cover-slip was ringed with vaseline, while in rabbit's blood they lived at 41 and 42° C. Petrie (1904) reported that he had kept mobile trypanosomes in a moist chamber at 37° C. in the dark for 3 days, while they remained alive on ice for a month, after which the mobility of the trypanosomes was found to be unchanged. Ashworth *et al.* (1908–9) were able to keep this trypanosome at 37° C. from 1 to 3 days, whereas in tubes kept in the ice-chest at −8° C. the organisms were actively motile for more than 1 week.

I had removed blood aseptically from the ear vein of rabbits. Two tubes of this infected blood were kept in the ice-chest, two at 37° C., two at 28° C. and two at room temperature (18–20° C.), as well as many fresh preparations with cover-slips ringed with vaseline. It was found that the trypanosomes did not survive more than 3–4 days in the ice-chest, while in all other cases they did not remain alive for more than 24 hr.

Development in culture

Petrie (1905) succeeded in cultivating the wild-rabbit trypanosome on rabbit's blood agar, in which forms similar to *T. lewisi* developed. After the sixteenth day, when the tubes were kept at room temperature, he could see some dividing forms though not in great numbers. Channon & Wright (1927) failed to cultivate the trypanosome in N.N.N. and W.N. media at room temperature in the dark.

I had also tried unsuccessfully to cultivate blood from the wild rabbits, as well as from the artificially infected rabbits, in N.N.N., W.N. and Hartley's Broth

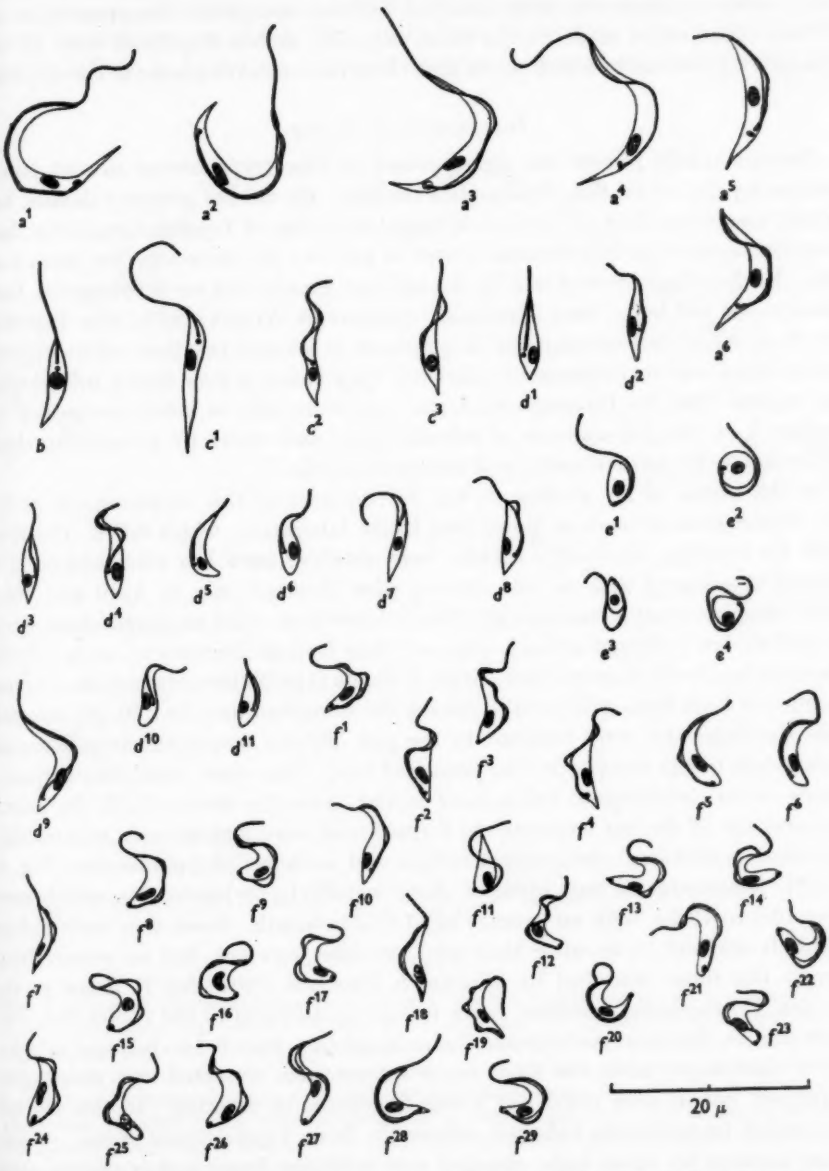

Fig. 8. Developmental forms of *T. nabiasi* in the gut of the rabbit flea, *Spilopsyllus cuniculi*. a^1–a^6, blood forms from the foregut of the flea within 24 hr. of the feed; *b*, leptomonad form; c^1–c^3, long crithidial forms in the gut, 3 days after infective feed; d^1–d^{11}, short stumpy transitional piriform individuals from rectum of the flea; e^1–e^4, leishmanial forms; f^1–f^{29}, metacyclic trypanosomes from the rectum of naturally infected fleas.

Digest media. In a few N.N.N. medium tubes, examined 5–6 days after inoculation, mobile trypanosomes were observed, but they completely disappeared on the second examination made on the tenth day. The mobile flagellates were all unchanged trypanosomes which shows that there was no development in the medium.

Development in the flea

Brumpt (1913) proved the development of this trypanosome in and transmission by the rabbit flea, *Spilopsyllus cuniculi*. He did not give any details, but simply mentioned that the cycle is identical with that of *Trypanosoma lewisi*, and that the metacyclic trypanosome forms of the two trypanosomes are similar in size. No flagellates were found in the salivary glands and no development took place in the bed bug, *Cimex lectularius*. Channon & Wright (1927), who dissected 120 fleas, found various stages of development in seventy-two fleas, while in forty others there was no evidence of infection. They found a very heavy infection in the rectum with the trypanosome forms, and were able to infect one rabbit by feeding it on the gut contents of infected fleas, and others by inoculating them subcutaneously, intravenously and intraperitoneally.

In the course of my studies on the development of this trypanosome in the flea, attempts were made to breed fleas in the laboratory, which failed. The fleas used for infecting laboratory rabbits were obtained from the wild rabbits. The highest numbers of fleas on wild rabbits were obtained only in April and May, after which on many occasions only two or three fleas could be caught from three to four rabbits collected on each trip, and thus further attempts to work out the life cycle had to be discontinued. Allan & Shank (1955) also obtained the highest number of fleas from wild rabbits during the same two months. In the infected fleas the flagellates were confined to the gut. As the metacyclic trypanosomes were found in the rectum, it was presumed that—like other *lewisi*-like trypanosomes—their development takes place in the 'posterior station'. In the smear preparations of the gut contents the forms found were leptomonad, leishmanial, crithidial, transitional piriform individuals and metacyclic trypanosomes (Fig. 8, a^1–f^{29}). Measurements were made of thirty metacyclic trypanosomes, which were from 10·0 to 13·2μ, with an average of 11·87μ in length. Some fleas were fed on rabbit R 500, and 24 hr. after their feed, sections were cut, but no intracellular forms—like those described by Minchin & Thomson (1915) for *T. lewisi* in the rat flea, *Ceratophyllus fasciatus*—were found. In addition to the rabbit flea, two more insects, *Rhodnius prolixus* and *Cimex lectularius*, were fed on infected rabbits. Forty-eight hours after the feed, many insects were dissected and thoroughly examined, but in none could any viable flagellates be detected. In this respect the rabbit trypanosome behaved differently from *Trypanosoma lewisi*, which, when ingested by these bugs, changed into crithidial forms and remained alive for 10 and 7 days respectively in the gut of the bugs.

The successive stages of development of *T. nabiasi* in its final and intermediate hosts, described in this paper, are represented in the accompanying figures (Figs. 8, a^1–f^{29}; 9, *b*–*l*).

DISCUSSION

From the observations of Roudsky (1910, 1911), Delanoë (1911), Coventry (1929) and Taliaferro & Pavlinova (1936), it appears that most of the *lewisi*-like try-

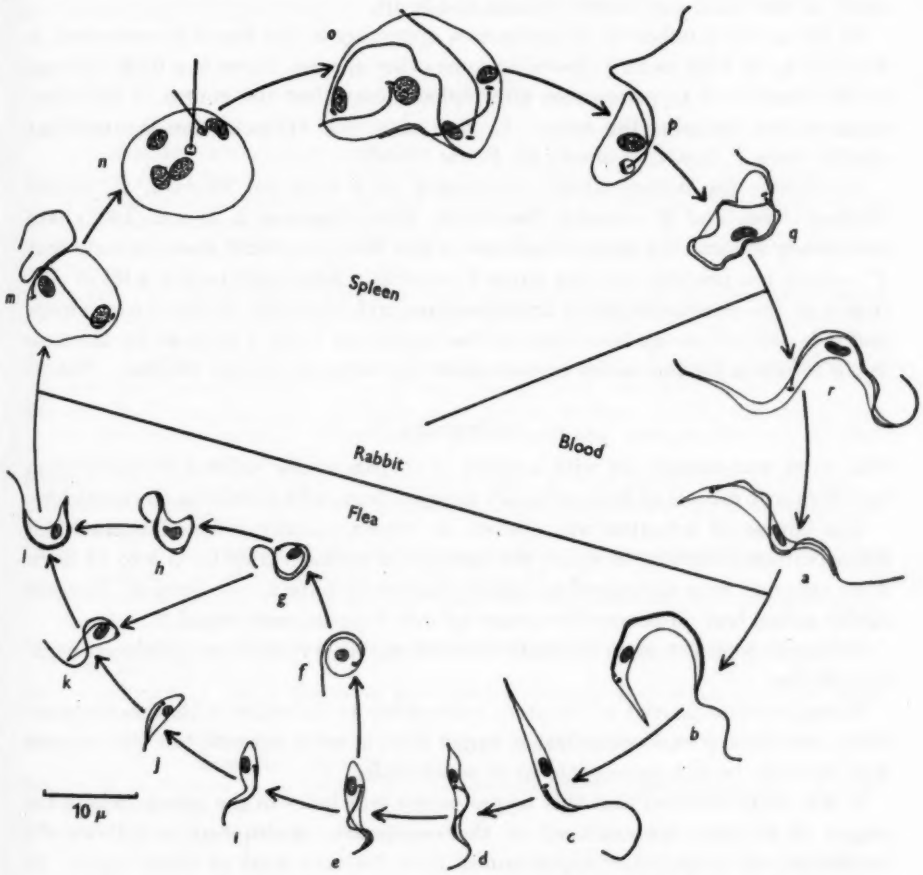

Fig. 9. Diagram of the probable life cycle of *T. nabiasi* in rabbit and flea. *a*, adult trypanosome in the peripheral blood of rabbit; *b*, trypanosome undergoing developmental changes after ingestion by flea; *c*, long crithidial form in the foregut; *d*, *e*, *i*, *k*, transitional piriform individuals in the hindgut; *f*, *g*, *h*, transformation into leishmanial forms, giving rise to *l*; *l*, metacyclic trypanosome; *m–o*, reproductive (division) stages from the blood capillaries of the spleen of rabbit; *p*, *q*, developmental forms with trypanosome-like arrangement, from the spleen, giving rise to blood forms (*r*, *a*); *r*, an unusually large form from the peripheral blood of rabbit seen at the time of division.

panosomes of rodents represent strains or races of *T. lewisi*. The relationship of the trypanosome of rabbits to *T. lewisi* is not the concern of this paper. In the present work it has been demonstrated that the development of the rabbit trypanosome takes place in the capillaries of the spleen. In this connexion it is

interesting to note that recent observations by Dr W. E. Ormerod and Mr R. Killick (unpublished) in this Department have clearly shown the presence of division stages of *T. lewisi* in the vasa recta of the kidney of the rat, while Davis (1952) has described the division stages of *T. zapi*, another *lewisi*-like trypanosome, in the blood capillaries of lungs and heart.

As far as the number of trypanosomes appearing in the blood is concerned, it does not go as high as in *T. lewisi* or some other species. There is a little increase in the number of trypanosomes after splenectomy, but the course of infection, more or less, remains the same. In size, also, this trypanosome is somewhat smaller than *T. lewisi*, as shown by Petrie (1904).

As regards the nomenclature, two names have been put forward, *T. nabiasi* Railliet, 1895, and *T. cuniculi* Blanchard, 1904. Laveran & Mesnil (1907) have completely ignored the name *T. nabiasi*, while Wenyon (1926) stated clearly that *T. nabiasi* has priority over the name *T. cuniculi*. Although there is a lot of confusion in the nomenclature of trypanosomes and especially in the *lewisi* group, and it is difficult to say how many of the names are valid, I propose for the time being to retain for the rabbit trypanosome the name *T. nabiasi* Railliet, 1895.

SUMMARY

This work was carried out with a strain of trypanosomes isolated by inoculation into domestic rabbits of fleas naturally infected from wild rabbits in Hertfordshire.

The course of infection was studied in experimentally infected normal and splenectomized rabbits, in which the incubation period varied from 5 to 12 days. After recovery from the infection, rabbits became immune to re-infection. Immune rabbit serum had no protective action against *Trypanosoma lewisi*.

Attempts to infect other animals with the rabbit trypanosome produced negative results.

Young rabbits proved to be more susceptible to infection with this trypanosome, and their parasitaemia lasted longer than in adult rabbits, but the parasite was found to be non-pathogenic to these animals.

It was demonstrated that this trypanosome multiplies in the spleen, where the stages of division are confined to the capillaries. Multiplication follows the inoculation of metacyclic trypanosomes from infected fleas or blood forms. It proceeds by division of the nucleus and kinetoplast into four, followed by fission of the cytoplasm, giving rise to four small daughter individuals. These are transformed into trypanosome forms, which are shed into the peripheral blood, where they change into the typical adult trypanosomes.

The development in the intermediate host takes place in the gut of the rabbit flea, *Spilopsyllus cuniculi*, with the production of metacyclic trypanosomes in the hind-gut (posterior station).

The nomenclature and synonymy of the rabbit trypanosome are discussed, and it is concluded that its valid name is *Trypanosoma nabiasi* Railliet, 1895.

I wish to express my sincere gratitude to Prof. P. C. C. Garnham of the Department of Parasitology, London School of Hygiene and Tropical Medicine, for his

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LERNAEID COPEPODS PARASITIC ON FLYING FISH

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(With 26 Figures in the Text)

Species of the genus *Pennella* are of interest because they parasitize widely different hosts such as cetacea, flying fish, pipe fishes, sword fishes, etc. In 1917 Wilson listed fourteen valid species and nine more have been added since. Of these *P. exocoeti* (Holten) and *P. blainvilli* (Le Sueur) have been recorded as parasites of flying fishes, the former probably in *Exocoetus speculiger* from which Leigh-Sharpe (1928) and Debouttéville & Nunes-Ruivote (1954) recorded the species, while the latter was found on *E. volitans*. The present paper deals with four parasites collected from four different species of flying fishes of Madras. The types will be deposited in the Indian museum, Calcutta.

Pennella robusta n.sp. (Figs. 1–6)

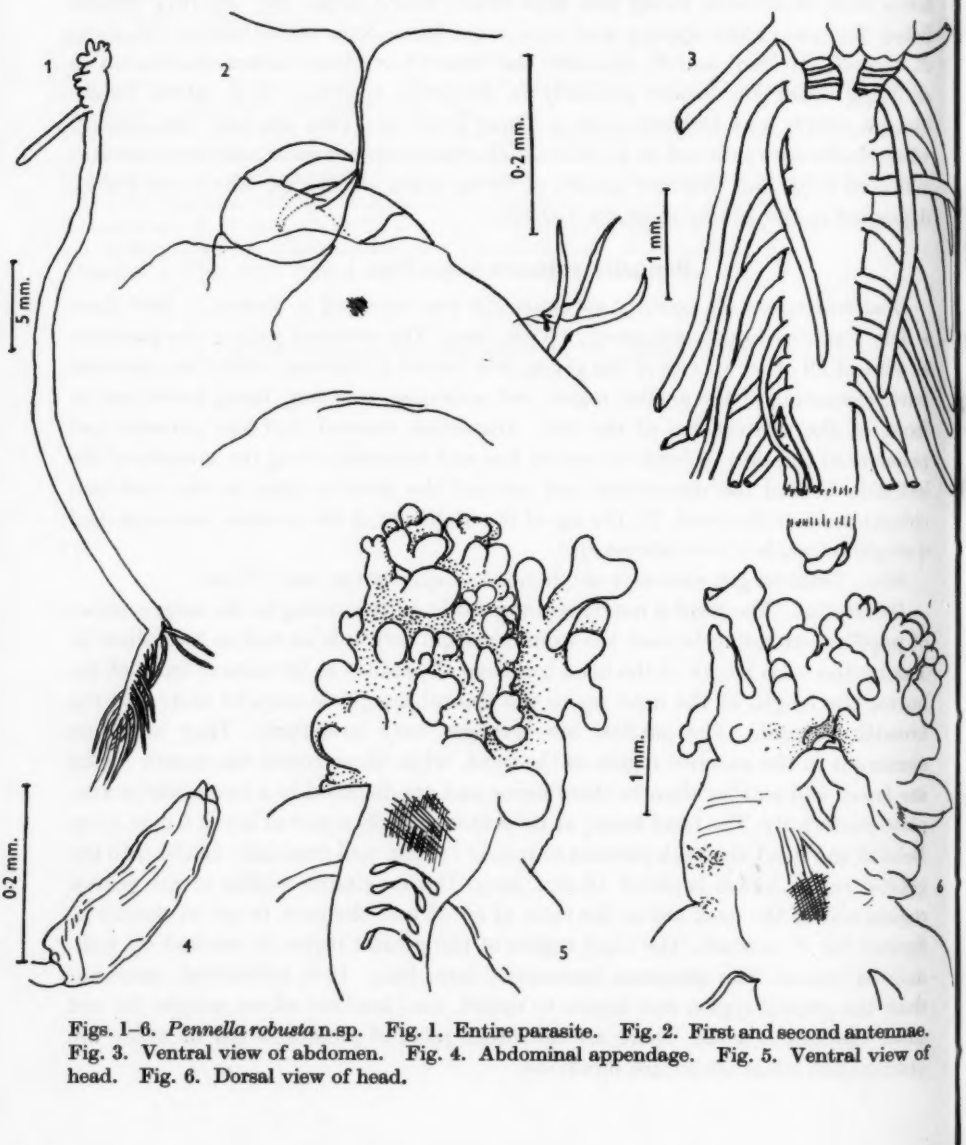
Host and record. A solitary specimen (♀) was obtained in February 1950 from a fish, *Paraexocoetus brachypteris*, 9·5 cm. long. The anterior part of the parasite, as that of all other species of the genus, was buried in the host, while the posterior part, consisting of the genital region and abdomen, was free, being found just in front of the ventral fins of the fish. Dissection showed that the parasite had penetrated the skin in front of ventral fins and extended along the muscles of the left side behind the operculum and reached the muscles close to the vertebral column behind the head. To the tip of the abdomen of the parasite was attached a single barnacle (*Conchoderma* sp.).

Size. Total length exclusive of plumes and egg strings was 5·5 cm.

Description. The head is nearly rectangular in outline owing to the large number of papillae extending beyond the frontal margin forwards as well as lateralwards. Taking the total length of the head inclusive of papillae as 90 units in front of the horns, the length of the head up to the frontal margin is only 55 units and the breadth 55 units. The papillae are arranged very irregularly. They are more numerous in the anterior region of the head, while those round the mouth region are fewer and smaller than in other forms and are disposed in a semicircle incomplete posteriorly. The head bears, at its posterior limit, a pair of horns 5 mm. long. Behind the head the neck extends to nearly 18 mm. and gradually dilates into the genital region which is about 15 mm. long. The maximum widths of the genital region and of the neck are in the ratio of 50:38 and the neck is not so slender as figured for *P. exocoeti*. The hind region of the genital region is marked by well-defined vulvae. The abdomen measures 7 mm. long. It is cylindrical, narrower than the genital region and tapers to tumid, anal laminae whose margin did not show vestiges of setae. There are seventeen pairs of plumes, a few of which are unbranched while others are biramous.

Appendages. The first antenna is greatly shortened, but is composed of three articles, each of which bears a long spine. The second antenna also is shortened and therefore appears broad. The terminal of three joints is a chela. There are four pairs of abdominal appendages arranged on the ventral side of the neck close behind the cephalic horns. Each appendage is conspicuous only by the basipod, which is large and elliptical. The two rami are represented by two short, pointed, toothlike structures.

Taxonomic remarks. It is unfortunate that though *P. exocoeti* has been described by Holten (1802), Desmarest (1825), Steenstrup & Lutken (1861), Charcot (1912),



Figs. 1–6. *Pennella robusta* n.sp. Fig. 1. Entire parasite. Fig. 2. First and second antennae. Fig. 3. Ventral view of abdomen. Fig. 4. Abdominal appendage. Fig. 5. Ventral view of head. Fig. 6. Dorsal view of head.

Leigh-Sharpe (1928), as well as Debouttéville & Nunes-Ruivo (1954), yet details of appendages of this species parasitic on flying fish are lacking. Host specificity cannot be stressed too far, because as Fage (1931) has pointed out, *P. filosa* occurs on different hosts and recently Weber & Beaufort (1922) have revised the genus *Exocoetus* and have classified the flying fishes into four genera. *E. mento*, the host fish of the present parasite, is now named *Parexocoetus brachypteris*. The parasite, which differs from all the known species, is therefore named, without reference to the hosts, *P. robustus*, the name signifying that it is more or less stout throughout its whole length. This species can be distinguished by the shortened three-articled first and second antennae, the general rectangular form of the head, the neck being longer than the genital segment and more than twice the length of the abdomen but slightly thinner than the genital segment. Other distinguishing features are the irregular disposition of the papillae and the simple two-branched plume.

Pennella platycephalus n.sp (Figs. 7–13)

Host and record. A single parasite (♀) was taken from *Cypsilurus spilopterus* by Mr Bashiruddin of the Central Marine Fisheries, to whom the author is thankful for the gift of this specimen.

Size. Exclusive of plumes and egg-strings, it measured 37 mm. long.

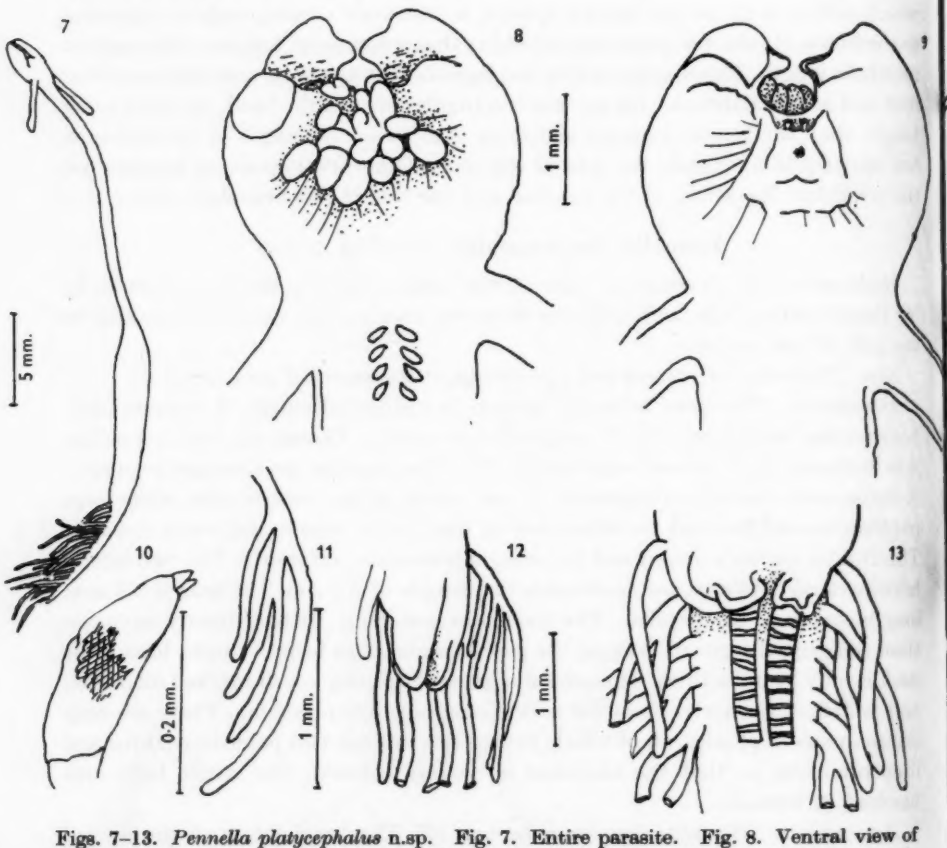
Description. The head is nearly circular in outline. Medially it appears cleft, because the two lateral sets of papillae do not overlap. Though the head is swollen, it is flattened on the dorsal and ventral sides. The papillae are arranged regularly. A dense oral cluster is conspicuous in the middle of the ventral side, while large papillae extend forward on either side of the frontal region and curve inwards. The frontal region is raised and the margin is concave anteriorly. The two lateral horns extend outwards and backwards to a length of 3·5 mm. The head is 3·5 mm. long inclusive of the papillae. The neck is 14 mm. long. It is distinctly narrower than the genital segment. Taking the genital segment as being 54 units broad, the neck is only 30 units thick. The genital segment begins by a well-marked dilatation and is definitely shorter than the neck, being only 100 mm. long. There are only sixteen pairs of plumes, all of which, except the anterior two pairs, have three and four branches, so that the abdomen is densely covered. The entire body was blackish in formalin.

Appendages. The antennae were broken off. The basal joints of the second antennae were set close together directed forwards. There are four pairs of abdominal appendages, arranged on the ventral side of the anterior end of the neck. Each consisted of a long elliptical basipod bearing two short stout spines—the remnants of the two rami.

Taxonomic remarks. That this form belongs to a species distinct from *Pennella exocoeti* and the three others described in this paper, is evident from its relatively shorter, stouter, genital segment, its many-branched plumes, the circular cushion-like but dorso-ventrally flattened head and the regular arrangement of the papillae. The fact that it was parasitic on a different host—*Cypsilurus spilopterus*—is also significant.

Pennella elegans n.sp. (Figs. 14–19)

Host and record. A single specimen (♀) was obtained by Mr Bashiruddin from a species of *Cypsilurus*, different from *C. spilopterus*, but which has some features of *C. oxycephalus* and of *C. altipennis*. It is believed that this fish may be a new species or subspecies.

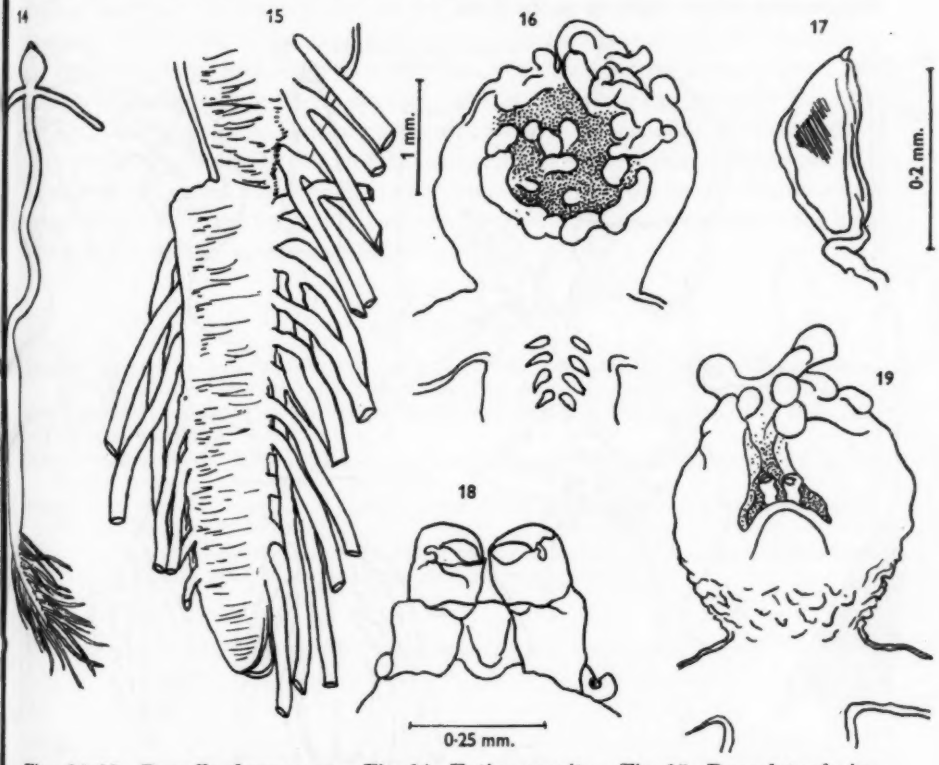


Figs. 7–13. *Pennella platycephalus* n.sp. Fig. 7. Entire parasite. Fig. 8. Ventral view of head. Fig. 9. Dorsal view of head. Fig. 10. Abdominal appendage. Fig. 11. A single plume. Fig. 12. Hind-end of abdomen. Fig. 13. Anterior end of abdomen.

Size. The parasite was 35 mm. long exclusive of egg strings and plumes.

Description. The cephalic horns are long (6 mm.) while the rest of the head is relatively small. The head is nearly 4 mm. long with an anterior median cleft extending to the middle of its length where the frontal margin occurs. The entire anterior half of the head is occupied by the papillae. On the dorsal side the papillae are larger and fewer and laterally arranged, but ventrally they are smaller and cross over the median line to become generally mingled with the oral circle of papillae, in such a way that only a narrow outer fringe of the ventral surface of the head is free of papillae. The neck is 15 mm. long and is about 1·5 mm. stout and leads

into a relatively shorter genital segment 11 mm. long and 2 mm. stout. The vulvae are well marked and placed close together. The abdomen is only 5·5 mm. long. It is attached to the genital segment by a slightly constricted anterior part. There are nearly eighteen plumes on each side. Except the very first, which starts from the hind end of the genital segment, the rest are bifurcate, one ramus being far shorter than the other in a few of the plumes.



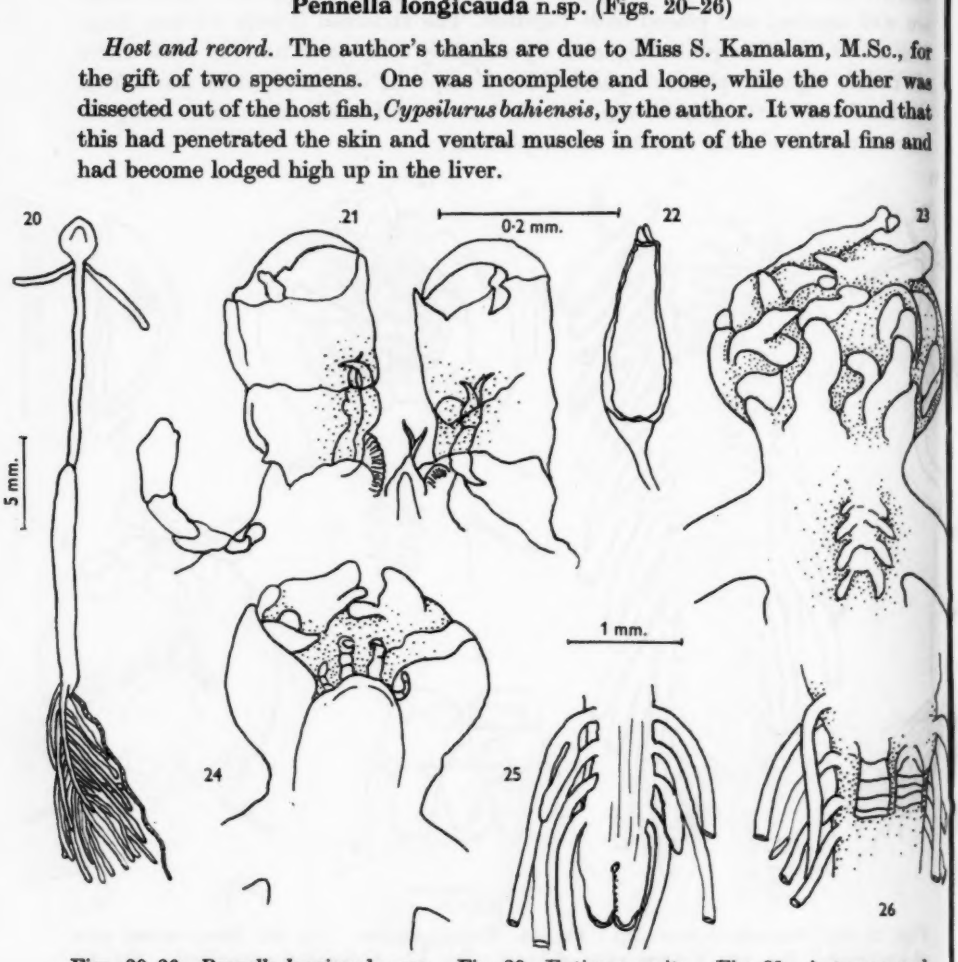
Figs. 14–19. *Pennella elegans* n.sp. Fig. 14. Entire parasite. Fig. 15. Dorso-lateral view of abdomen. Fig. 16. Ventral view of head. Fig. 17. Abdominal appendage. Fig. 18. Second antennae. Fig. 19. Dorsal view of head.

Appendages. The first antennae had been removed while cleaning with a camel-hair brush. The second antennae were large and projected forwards from the frontal margin. They are three-jointed, chelate. The chela folds against a well-marked prominence on the second joint. The paired thoracic appendages, four in number, are attached so far in the front region of the neck that the first two were between the bases of the cephalic horns. Each has a long elliptical basipod of somewhat triangular outline, bearing two claw-like remnants of the rami.

Taxonomic remarks. The long cephalic horns and the general shape of the head, size and disposition of the papillae, the form of the antennae and thoracic appendages, the shape of the abdomen, as well as the number and type of plumes, mark this species as distinct from others.

Pennella longicauda n.sp. (Figs. 20–26)

Host and record. The author's thanks are due to Miss S. Kamalam, M.Sc., for the gift of two specimens. One was incomplete and loose, while the other was dissected out of the host fish, *Cypsilurus bahiensis*, by the author. It was found that this had penetrated the skin and ventral muscles in front of the ventral fins and had become lodged high up in the liver.



Figs. 20–26. *Pennella longicauda* n.sp. Fig. 20. Entire parasite. Fig. 21. Antennae and maxillae. Fig. 22. Abdominal appendage. Fig. 23. Ventral view of head. Fig. 24. Dorsal view of head. Fig. 25. Hind-end of abdomen. Fig. 26. Front end of abdomen.

Size. The total length exclusive of egg strings and plumes was 35 mm.

Description. The head is circular and flattened. It measures 3·5 mm. across, with the two horns projecting obliquely backwards for about 5·5 mm. The entire ventral surface is covered by papillae overlapping each other irregularly. On the dorsal side a raised median frontal region can be distinguished from two broad lateral areas from which papillae curve inwards so closely that there is no deep median cleft as in *Pennella platycephalus*. The neck is distinct and measures about 11·5 mm. It is thinner than the genital segment, which is 12·5 mm. long. The vulvae are so close together that the genital strings touch each other. The abdomen is relatively long, being 75 mm., and tapers to a narrow truncate tip. The anal

laminae bear a short spine each. The plumes are about twenty-two in number on each side, most being forked into two rami.

Appendages. The first antenna is five-jointed and is attached to the frontal margin, more lateral and external than the second antenna. The terminal joint is broad and blunt. There were no spines or setae. The second antennae are larger, stouter and chelate. They are three-articled. They project straight from the frontal margin. Between them can be seen, when cleared in xylol, the more ventrally placed first and second maxillae. The second maxilla appears biramous, the basipod is large and bears an endite-like lobe. Each ramus is two-jointed, the distal joint being a claw. There are four pairs of abdominal appendages. Each leg has a more or less oblong basipod and bears two rami reduced to stout short spines.

Taxonomic remarks. The shape of the head and disposition of the papillae especially on its ventral surface, the form of the antennae, the relatively longer abdomen and its uniformly tapering form, and the large number of plumes attached to the long abdomen, mark this species as distinct from the others.

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CERCARIA DIPLOSTOMI PHOXINI (FAUST), A FURCOCERCARIA WHICH DEVELOPS INTO *DIPLOSTOMULUM PHOXINI* IN THE BRAIN OF THE MINNOW

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(With Plate XI and 13 Figures in the Text)

A previous paper (Rees, 1955) described a very heavy infestation of *Diplostomulum phoxini* (Faust) in the brain of large numbers of minnows (*Phoxinus phoxinus* (L.)) from Fron Goch Pool near Aberystwyth. The diplostomulum stage was described and the adult worm obtained by feeding infested minnows to ducklings. The adult worm should be referred to as *Diplostomum phoxini* (Faust, 1918) syn. *Tetracotyle phoxini* Faust, 1918, *Diplostomum pelmatoides* Dub., 1932; Rees, 1955. In an attempt to complete the life cycle large numbers of molluscs were examined at the same time from the same pool but the cercariae were not found. The search, however, was continued and in August 1955 the sporocyst and cercaria were eventually discovered in *Lymnaea peregeri*.

While the first part of this investigation was in the press a paper appeared by Arvy & Buttner (1954) on the life cycle of *Diplostomum phoxini*. Recent papers on other aspects of this interesting worm are those of Arvy (1954) and Arvy & Buttner (1955). Working in Paris, Arvy & Buttner (1954) found that *Lymnaea auricularia* (L.) harboured furcocercariae which when placed in contact with *Phoxinus laevis* (= *P. phoxinus*) penetrated rapidly into the fishes and within 48 hr. at the latest reached the brain where they developed into *Diplostomulum phoxini*. They later obtained specimens of the adult worm on feeding infested minnows to *Cairina moschata* L. var. *domestica* and *Anas boschas* L. Similar experiments have been carried out (Rees, 1955) using very young individuals of the domestic duck, *Anas platyrhynchos* L. var. *domesticus* (= *Anas boschas*). The life cycle of this worm, therefore, has been demonstrated experimentally and independently by Arvy & Buttner and the writer. The description of the cercaria given by Arvy & Buttner is very brief and so it is described fully below. Some account is also given of the early stages of infestation in the minnow.

MATERIAL AND METHODS

The only species of mollusc obtained from Fron Goch Pool was *Lymnaea peregeri* (Müll). Large numbers were collected from the shallow water around the margin where they occurred very abundantly on the muddy bottom or attached to stones. The molluscs were brought back to the laboratory and isolated at first in groups in small aquaria and later, when cercariae appeared in the water, singly, to determine which specimens were infected. In spite of the very large number examined only a few, namely 3·9 %, were found to be parasitized by *Cercaria diplostomi phoxini*

and these were identified as *Lymnaea pereger* var. *ovata* (Drapanaud). Arvy & Buttner (1954) examined thirteen species of gastropods, but found this cercaria only in *L. auricularia* (L.), a species which does not occur in Fron Goch Pool. The first intermediate host, therefore, is different in Britain and France. Dr C. A. Hopkins, of the Department of Zoology in the University of Glasgow, in a letter to me, states that he, also, has found the cercaria in *L. pereger* in Scotland. The fact that so few molluscs are parasitized is surprising, as every minnow examined from the pool over a considerable period was found to be infested to varying degrees with the diplostomulum stage. Arvy & Buttner obtained a very similar result recording an infestation of 4–5 % in *L. auricularia* in a region where all the minnows were parasitized.

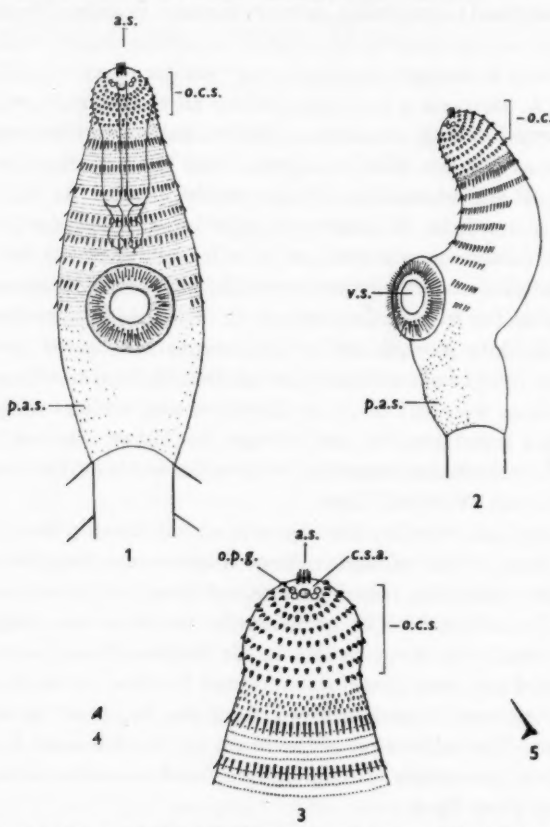
Cercariae emerged in large numbers in the laboratory and were examined alive in water as soon as possible after emergence from the snail host, as only in such specimens were all the anatomical details clearly visible. As the cercaria is an extremely small one, a $\frac{1}{12}$ in. oil-immersion objective was essential for examining its structure. Some measurements were made on living specimens, but owing to the great power of extension and contraction of the body such measurements are not sufficiently reliable for specific determination. Specimens were fixed rapidly by dropping into absolute alcohol and measurements were made from these. Two infected molluscs were removed from their shells and fixed in Gilson's fixative for sectioning. Sections were cut at 5μ in thickness and stained with Heidenhain's azan or Ehrlich's haematoxylin and orange G. Other infected molluscs were removed from their shells and teased apart in saline to obtain the sporocyst which, like the cercaria, was examined alive.

In order to ascertain whether this cercaria would develop into *Diplostomulum phoxini* in the brain of the minnow a large number was transferred in water to a small aquarium containing minnows obtained from a different source where no specimens had been found to be parasitized. Controls were kept. The actual infection experiment was done by Mr L. E. Hughes, Veterinary Investigation Officer, to whom I am most grateful. As found by Arvy & Buttner (1954) large numbers of larvae were later recovered from the brain of the experimentally infested minnows. The infestation of the brain will be discussed later and will be compared with an infestation of long standing such as is found in minnows collected from Fron Goch Pool.

Cercaria diplostomi phoxini

On emergence from the snail host the cercariae swim rapidly and spasmodically in any direction. Agitation of the water will start them moving, but they soon come to rest hanging downwards in the water with the furcae wide apart, each forming an angle of about 135° with the tail stem. The body is exceedingly contractile, and when fully extended is of uniform diameter along its length. When at rest and when contracted it is widest in the region of the ventral sucker. It assumes this shape, also, on fixation (Text-figs. 1, 6–8). The ventral sucker is just behind the middle of the body and there are no pigment patches. The tail stem is fairly broad, longer than the body and bifurcates to form two caudal furcae which taper to their extremities and are slightly shorter than the tail stem (Text-fig. 7). The measure-

ments in mm. given below are taken, with one exception, from fixed specimens. They differ slightly from those given by Arvy & Buttner (1954) but, as already stated, dimensions of such contractile specimens are not reliable for specific diagnosis: Body length, 0·125 (fixed), 0·22 (living and fully extended); body breadth, 0·041; tail stem length, 0·200; tail stem breadth, 0·024; tail furca length, 0·175; ventral sucker diameter, 0·027; pharynx diameter, 0·012.



Text-fig. 1. *Cercaria diplostomi phoxini* (Faust), ventral view of body showing arrangement of spines.

Text-fig. 2. Lateral view showing arrangement of spines on the dorsal and ventral surfaces.

Text-fig. 3. Ventral view of anterior extremity showing oral cap and neighbouring spines in detail.

Text-fig. 4. Single spine from oral cap.

Text-fig. 5. Single spine from ventral sucker.

Key to lettering, p. 137.

The mouth is slightly subterminal on the ventral surface (Text-figs. 1–3, 6–8) and is surrounded by a narrow circum-oral spineless area (Text-figs. 1–3). In this area on either side of the mouth are two openings of the ducts of the penetration gland cells. Immediately in front of the mouth is a group of five spines which point

directly forwards and occupy the anterior tip of the body (Text-figs. 1–3). These spines are strong and straight, each tapering to a sharp point. The circum-oral spineless area is followed by an oral cap of spines extending backwards to a level of one-third of the length of the oral sucker. It consists of seven alternating transverse rows of stout backwardly directed spines with broad bases (Text-figs. 1–4). Following the oral cap and encircling the body is a very narrow area devoid of spines. In living specimens the anterior tip of the worm, that is that bearing the oral cap of spines, is constantly drawn in or invaginated and thrust out again with considerable force, the spines of the oral cap then turning backwards and the apical group of spines projecting freely at the anterior extremity. This movement would presumably greatly assist the cercaria in penetrating the skin of the second intermediate host and burrowing through its tissues. The apical spines would probably puncture the skin and the spines of the oral cap maintain a firm hold on the margin of the initial perforation while the cercaria burrows its way into the body. Following the spineless zone which is immediately behind the oral cap are four irregular rows of very small straight spines encircling the body. Behind these the arrangement of the body spines differs on the dorsal and ventral surfaces. On the ventral surface there are eight transverse rows of large spines extending to a level nearly half way along the ventral sucker. The first five rows are complete ventrally, there being about twenty-four spines in each row (Text-fig. 1). The remaining three rows are incomplete in the mid-ventral line. Rows six and seven consist of about four to six spines on either side and row eight of only two or three. This separation of the spines into two ventro-lateral groups is due to the presence of the large ventral sucker. On the dorsal side only the first two rows of spines are complete (Text-fig. 2). The first two rows therefore encircle the body, but the spines on the dorsal surface are slightly smaller. The remaining six rows of spines extend slightly on to the dorsal surface, most of which, however, is devoid of spines. This is as would be expected, as it is the ventral surface which is in contact with the second intermediate host during penetration. The two complete rows of spines at the anterior end may assist in penetration as they follow quite closely behind the oral cap. Behind the ventral sucker there are no spines on the dorsal surface (Text-fig. 2), but small scattered spines are present ventrally except for a narrow area along the mid-ventral line immediately behind the ventral sucker (Text-fig. 1). In the cuticle between each transverse row of spines in the anterior half of the body are four rows of vertical striations which are best seen when viewed in profile (Text-figs. 1, 3). These are almost like very small spines which do not penetrate the surface and are probably the tiny facets described by Mataré (1909), Ashworth & Bannerman (1927) and the writer (1955) for the diplostomulum and adult stages of this worm.

The ventral sucker is provided with a double row of alternating spines around the margin, there being thirty-six in each row (Figs. 1, 2, 5). Arvy & Buttner (1954) gave the number of spines as twenty-eight, but with an oil immersion a total of seventy-two have been counted in a fully expanded sucker in the present instance. The spines are long, sharply pointed and have broad bases (Text-fig. 5). The orientation of the spines depends on the state of protrusion or retraction of the

sucker. When retracted they are hardly visible as they point inwards towards the cavity, but when the sucker opens out and protrudes slightly from the surface the spines spread out all around the margin (Text-fig. 2) and presumably assist in the attachment of the cercaria to the fish host during the early stages of penetration.

The tail stem is provided with twelve hair-like projections or bristles on either side, evenly arranged from the root of the tail to the distal bifurcation (Text-fig. 7).

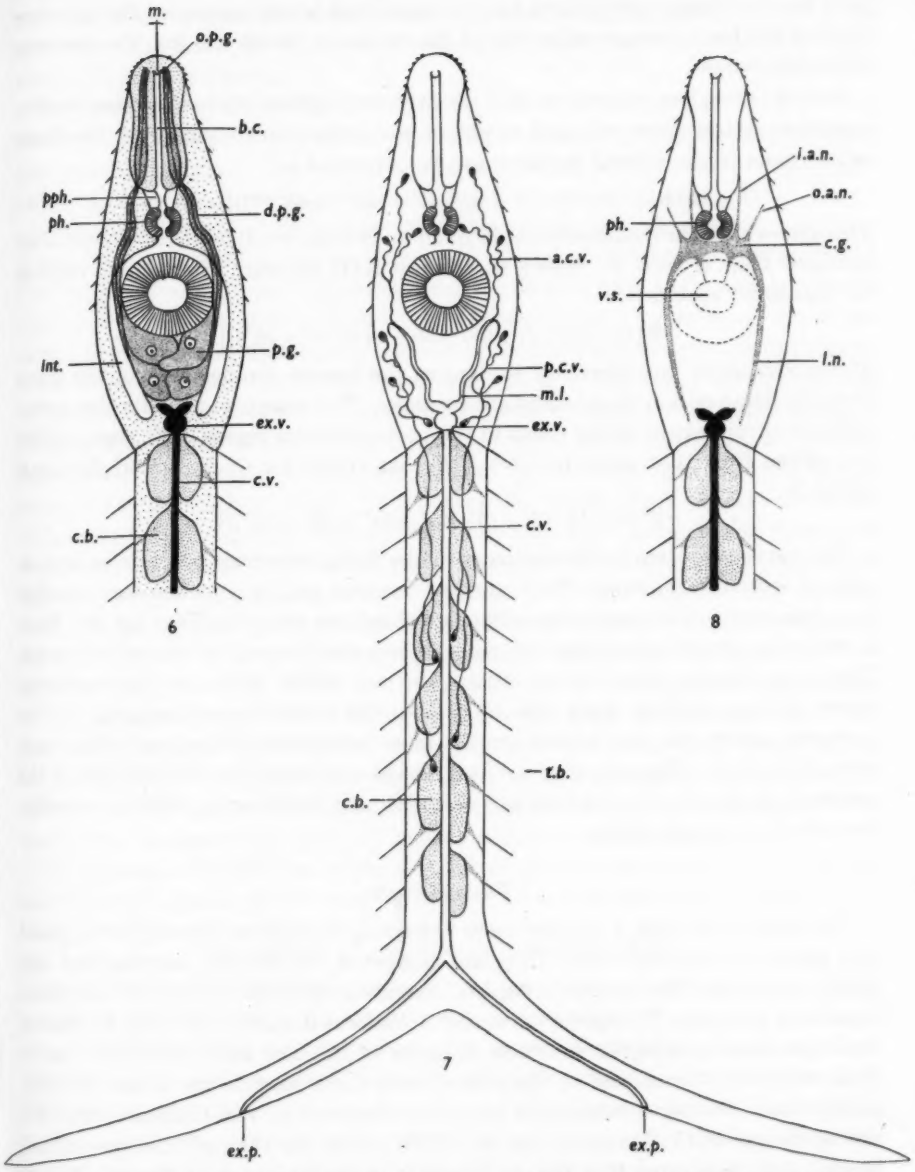
The slightly ventral mouth opens into a narrow tubular buccal cavity which passes through the oral sucker to open behind into a thick-walled prepharynx (Text-figs. 6, 9). This is followed by a muscular pharynx and short oesophagus (Text-fig. 6). The latter bifurcates in front of the ventral sucker, the intestinal caeca extending almost to the posterior extremity of the body. The caeca are dorsal in position, as can be seen in a transverse section through the post-acetabular region (Text-fig. 12).

There are four large penetration gland cells situated immediately behind the ventral sucker, in pairs, one behind the other in the ventral half of the body (Text-figs, 6, 12). Each has a large nucleus containing one nucleolus, the contents are granular. The ducts from the penetration-gland cells pass forwards, two on either side (Text-figs. 6, 10, 11), and enter the posterior margin of the oral sucker. There is a slight constriction in the ducts at their point of entry (Text-fig. 6), but they enlarge again as they traverse this organ (Text-figs. 6, 9). The ducts open by two pores on either side of the mouth in the circum-oral spineless area (Text-figs. 1–3, 6).

There are six pairs of caudal bodies in the tail stem which appear to be attached to the caudal excretory duct and also connected by strands to the wall of the tail (Text-fig. 7). The first pair lies immediately behind the tail root and the last pair a short distance in front of the forking of the tail stem. The arrangement of the caudal bodies is slightly irregular, the two members of a pair not being exactly opposite to one another. Hesse (1923) in describing *Cercaria* sp., suggested that caudal bodies may have an excretory function and may also give buoyancy to the cercaria.

The rounded excretory vesicle is situated at the posterior extremity of the body (Text-figs. 6–8). No 'island of Cort' has been observed. From the bladder the caudal excretory vessel passes down the centre of the tail stem between the six pairs of caudal bodies (Text-fig. 7). It bifurcates distally, one branch passing into each caudal furca to open on the adjacent sides of the furcae about half way along their length.

Into the anterior margin of the bladder open on each side the main lateral excretory vessels. They pass forwards without convolutions to just beyond the posterior margin of the ventral sucker (Text-fig. 7). There they receive, on either side, the anterior and posterior collecting vessels. Into the anterior collecting vessel on each side open the ducts from three flame cells and into the posterior collecting vessel the ducts from three flame cells in the postacetabular region and two in the tail stem. Of the two pairs of flame cells in the tail stem those on the left are a little farther forward than those on the right. The anterior flame cells of each pair are in the neighbourhood of the third pair of caudal bodies and the



Text-fig. 6. *Cercaria diplostomi phoxini* (Faust), ventral view showing alimentary canal and penetration glands.

Text-fig. 7. Ventral view of complete cercaria showing caudal bodies and excretory system.

Text-fig. 8. Dorsal view showing a reconstruction of the nervous system.

Key to lettering, p. 137.

posterior in the neighbourhood of the fourth and fifth. On either side the ducts from the two flame cells join to form a single duct which passes up the tail stem to enter the body, one on either side of the excretory bladder to join the posterior collecting tubule.

Dubois (1944) has pointed out that the excretory system of *Diplostomum* Nordm. comprises sixteen flame cells and no pre- or postacetabular commissures. The flame cell formula in the present instance can be expressed as

$$2[(1a+1b+2)+(3+4a+4b'+(4b''*+4b''**))]=16.$$

This agrees with the modified formula given by Dubois (1944) for *Cercaria diplostomi spathacei* Rud. (syn. *C. C.* Szidat, *C. helvetica* XIII Dubois). The original formula for this latter species was

$$2[(1a+1b+2)+(3a+3b+4a+(4b'+4b''))].$$

The modification was necessary to express the precise arrangement of the ducts from the flame cells in the postacetabular region. This amended formula also agrees with the arrangement of the ducts in the postacetabular region of *C. tenuis* Miller but differs from that given by Cort & Brooks (1928) for *C. diplostomi flexicaudi* which is

$$2[(1a+1b+2)+(3+4a'+4a''+(4b'+4b''))].$$

The nervous system is not apparent in the living specimen but can be seen in stained sectioned material. There are two cerebral ganglia joined to one another by a transverse commissure immediately behind the pharynx (Text-fig. 8). Each is somewhat irregular in shape and gives off two anterior and two posterior nerves. The inner anterior nerve curves around the oral sucker while the outer anterior nerve diverges slightly from this to pass to the antero-lateral margin. Of the posterior nerves the two lateral are the more prominent. They pass along each side of the body. The two short ventral nerves arise from the ventral side of the cerebral ganglia one on each side and pass obliquely backwards, postero-ventrally, towards the ventral sucker.

The sporocyst

The sporocysts form a tangled mass extending throughout the digestive gland and gonad of the snail host. They are elongated, thread-like, unbranched and white in colour. The longest complete specimen dissected out from the gland measured 10·0 mm. The sporocyst is not of uniform diameter but may be dilated and constricted at irregular intervals along its length. The width, therefore, varies from 0·08 to 0·17 mm. and in the widest parts there may be up to six cercariae side by side. Similar constrictions have been observed by van Haitsma (1931) for the sporocyst of *C. flexicauda* and by Miller (1923) for that of *C. chrysenterica*. Hesse (1923) suggested that the constrictions in the sporocyst of *Cercaria* sp. are probably due either to the resistance of the tissues of the digestive gland or to incipient transverse division of the sporocyst. At one extremity the sporocyst tapers abruptly to form a fairly mobile tip and shortly behind the tip is a birth pore surrounded by a slight prominence. From this a short birth canal leads to the cavity of the sporocyst. The wall of the sporocyst (Text-fig. 13) consists of

a thin outer layer in which it was impossible to distinguish cell outlines. This is followed by a few delicate muscle fibres and a layer of cells lining the cavity of the sporocyst. The latter are best seen in young sporocysts and are then very large, each with a large nucleus.

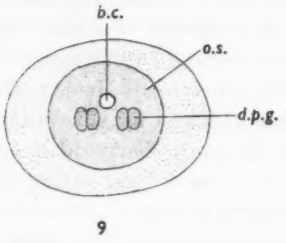
As noted by Arvy & Buttner (1954) this species of cercaria appears to differ from all other cercariae so far described. Seven other species of related cercariae have four penetration glands behind the ventral sucker and twelve caudal bodies in the tail stem. They are *C. furcicauda* Faust (1919), *Cercaria* sp. Hesse (1923), *C. micromorpha* Brown (1926), *C. laruei* Cort & Brooks (1928), *C. modicella* Cort & Brooks (1928), *C. chromatophora* Brown (1931) and *C. yogeni* Cort and Brackett (1937). The last two are easily distinguishable from the present form as they have pigment patches on the dorsal side in front of the ventral sucker. *C. furcicauda* Faust has twelve flame cells and there is only one pair in the tail stem. The penetration glands are more laterally placed and the furcae are at right angles to the tail stem. Hesse (1923) does not give an exact description of the arrangement of the body spines in *Cercaria* sp., but the ventral sucker has three rows of spines and there are fourteen flame cells. The ducts from the flame cells in the tail stem open into the caudal excretory duct, which is unique for this type of cercaria. This species also occurs in *Lymnaea pereger* and was recorded by Hesse from Loch Lubnaig and from Loch Vennachar in Perthshire. *Cercaria micromorpha* Brown is also a parasite of *Lymnaea pereger* in Britain. Brown (1926) described the body as being covered with extremely small sparingly distributed spines more closely set in the region of the oral sucker. The oesophagus and caeca are said to be absent and there are only ten flame cells. *Cercaria laruei* and *C. modicella* Cort & Brooks (1928) from species of *Lymnaea* in Douglas Lake, Michigan, also differ from the present form. They both have eighteen rows of spines on the body. The former has four rows of spines around the ventral sucker and a group of fifteen anterior spines, while the latter has ten anterior spines. The flame cell arrangement in *Cercaria laruei* is similar to that in the present form, but in *C. modicella* there are ten flame cells.

The cercaria of *Diplostomum phoxini*, therefore, although closely related to the above-named species, differs from all of them in several essential features.

Examination of experimentally infested minnows

As already stated, minnows were exposed to cercariae to ascertain if they would penetrate into these hosts. In the first instance a number of cercariae which had recently emerged from *Lymnaea pereger* var. *ovata* was placed in a small aquarium containing some minnows which were obtained from a lake which was free from the parasite. One of the minnows was removed 48 hr. later and killed by scattering crystals of eurythane into the water containing the fish. After death the fish was washed, decapitated, and the head examined in saline under a binocular. One of the controls was treated in the same way for comparison. On the removal of the roof of the skull of the minnow which had been exposed to cercariae it was obvious that there had been a certain amount of cerebral haemorrhage. The whole brain was faintly pink in colour, suggesting that minor haemorrhages had occurred throughout the brain tissue and blood had leaked into the ventricles and collected superficially

in the various fissures of the brain. No such haemorrhages or pink coloration was seen in the control specimen nor in the case of an infestation of long standing. Numbers of larvae were present in the cranial cavity of the infested fish and others were teased out of the brain tissue. The larvae had not, at this stage, accumulated



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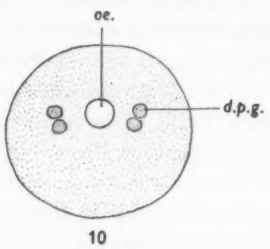

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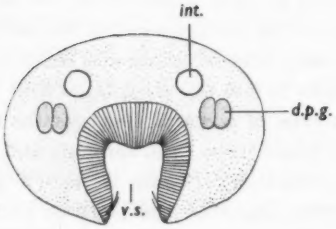

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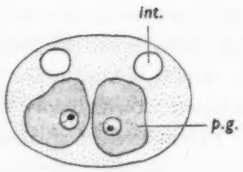

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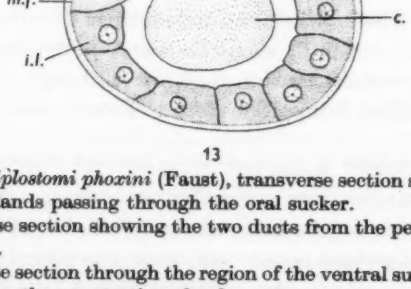

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Text-fig. 9. *Cercaria diplostomi phoxini* (Faust), transverse section showing buccal cavity and ducts of penetration glands passing through the oral sucker.
Text-fig. 10. Transverse section showing the two ducts from the penetration glands on either side of the oesophagus.
Text-fig. 11. Transverse section through the region of the ventral sucker showing the intestine dorsal to the ducts from the penetration glands.
Text-fig. 12. Transverse section through the postacetabular region showing the penetration glands lying ventral to the intestine.
Text-fig. 13. Transverse section through a young sporocyst showing the structure of the wall.

Key to lettering, p. 137.

in the ventricles so that none could be seen in a superficial examination of the brain itself. In infestations of long standing, as in minnows collected from Fron Goch Pool, the larvae could be seen in large numbers under the pia mater in the fourth ventricle and the whole brain is swollen in this region (Pl. XI, fig. 1).

Heads of other experimentally infested minnows were fixed for sectioning, 48 hr. after exposure to infection, in Bouin's fixative in which they were left for several weeks. Sagittal and transverse sections of the heads were cut at 10μ in thickness and stained with Ehrlich's haematoxylin and orange G. These sections were compared on the one hand with those of normal heads and on the other with those of heads where the infestation was one of long duration and in which the diplostomulum stage was fully developed.

On comparing a brain 48 hr. after infestation with an older infestation certain differences were immediately apparent. As already indicated by the writer (1955) in an old and heavy infestation the diplostomuli accumulate in the fourth ventricle, aqueductus sylvii, optic lobes, third ventricle, lobi inferiores, occasionally the corpora striata and under the epithelium covering certain areas of the brain and spinal cord. The diplostomuli are not free in the cavities of the brain but are actually under the epithelium lining the cavities. As this is raised up there is a proliferation of vacuolated tissue underneath it in which the worms lie. Fully developed diplostomuli are rarely found deeply embedded in the brain tissue. In a recent infestation, however, the opposite is the case. After 48 hr. the larvae have not reached the ventricles, which therefore appear to be empty (Pl. XI, figs. 2, 4 and 5). Sagittal and transverse sections show that the larvae are deeply embedded in the tissues of the brain and spinal cord. They can be seen around the fourth ventricle (Pl. XI, figs. 2, 4), in the medulla (Pl. XI, figs. 2, 5), and near the floor of the aqueductus sylvii and third ventricle. None was observed in the cerebellum and very few in the dorsal half of the brain. In the spinal cord (Pl. XI, fig. 3) they are again deeply embedded but show a tendency to accumulate near the central canal, on the dorsal side and around the roots of the anterior spinal nerves.

It seems reasonable to suppose that the larvae reach the brain by way of the blood stream. The pia mater is rich in blood vessels derived from internal carotid and vertebral arteries and in sections blood vessels can be seen to enter the brain and to penetrate deeply (Pl. XI, fig. 5). It is interesting to note that at this stage the larvae in the brain tissue are invariably found in groups around blood vessels (Pl. XI, fig. 6) and it seems probable that the larvae have emerged from them into the surrounding tissue. In one instance a larva was actually found in a blood vessel (Pl. XI, fig. 5). The suggestion that they reach the brain by way of the blood stream is supported by the fact that haemorrhages occur in the brain at this stage. Davis (1936) is of the opinion that the larvae of *Diplostomum flexicaudum* are carried from the skin of the tadpole, which they penetrate, to the brain very rapidly by way of the blood vessels. He has also observed them to enter the blood vessels of the tongue of a frog and to travel along them. Having emerged from the blood vessels the larvae presumably burrow their way through the tissues of the brain and spinal cord until they reach the epithelium lining the cavities or covering the surface. Pl. XI, fig. 4, shows a larva beginning to protrude into the fourth ventricle. The sagittal section (Pl. XI, fig. 2) also shows them accumulating near the ventricles.

Larvae which had recently entered the brain of the minnow had not advanced far beyond the cercaria stage. The body armature is unchanged and it seems that

the oral extremity is still capable of invagination and rapid evagination which action might assist the larva in burrowing through the brain. In Pl. XI, fig. 6, the oral cap of spines can be seen in two larvae, which lie near a blood vessel, and they appear to be thrusting their way through the surrounding tissue. The migration through the brain may be assisted by the secretion from the penetration glands which are still present. Having reached the internal or external surfaces of the brain or spinal cord the larvae continue their metamorphosis and when fully developed occupy the characteristic positions shown in Pl. XI, fig. 1. As stated by Arvy & Buttner (1954) the diplostomuli are fully developed in about 28 days after entry into the fish.

SUMMARY

1. A fork-tailed cercaria *Cercaria diplostomi phoxini* (Faust) has been found to develop in sporocysts in 3·9 % of *Lymnaea pereger* var. *ovata* (Drapanaud) in Fron Goch Pool near Aberystwyth.

2. The cercaria is described in detail. The arrangement of spines on the body is characteristic and there is a double row of seventy-two spines on the ventral sucker. The flame cell formula is given.

3. The cercaria is compared with seven closely related types.

4. Infestation experiments show that *Cercaria diplostomi phoxini* develops into *Diplostomulum phoxini* in the brain of the minnow.

5. The early stage of infestation of the brain of the minnow is described and compared with an infestation of long standing.

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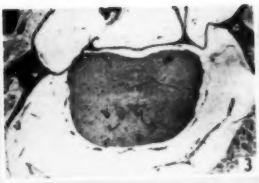

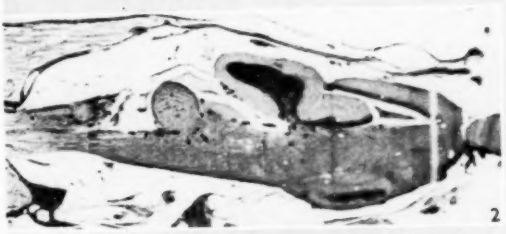

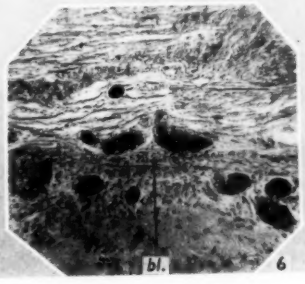

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KEY TO LETTERING OF FIGURES

(All drawings are semi-diagrammatic.)

a.c.v. anterior collecting vessel
a.s. apical spines
b.c. buccal cavity
c. cercaria
c.b. caudal body
c.g. cerebral ganglion
c.s.a. circumoral spineless area
c.v. caudal excretory vessel
d.p.g. ducts of penetration glands
ex.p. excretory pore
ex.v. excretory vesicle
i.a.n. inner anterior nerve
i.l. inner layer of sporocyst wall
int. intestine
l.n. lateral nerve
m. mouth
m.f. muscle fibres
m.l. main lateral excretory vessel
o.a.n. outer anterior nerve
o.c.s. oral cap of spines
o.l. outer layer of sporocyst wall
o.p.g. openings of ducts of penetration glands
o.s. oral sucker
oe. oesophagus
p.a.s. post acetabular spines
p.c.v. posterior collecting vessel
p.g. penetration gland
ph. pharynx
pph. prepharynx
t.b. tail bristle
v.s. ventral sucker

EXPLANATION OF PLATE XI

(Photomicrographs by J. E. Welbourn.)

Fig. 1. Sagittal section through the head of a minnow (*Phoxinus phoxinus* (L.)) showing a late stage of infestation with *Diplostomulum phoxini* (Faust).

Fig. 2. Sagittal section through the brain of a minnow, 48 hr. after exposure to *Cercaria diplostomi phoxini* (Faust), showing the larvae accumulating around the ventricles.

Fig. 3. Transverse section through the anterior end of the spinal cord of a minnow, 48 hr. after infection, showing larvae embedded in the tissue.

Fig. 4. Transverse section through the region of the fourth ventricle of the brain of a minnow, 48 hr. after infection, showing larvae around the ventricle and near the external surface.

Fig. 5. Transverse section through the anterior end of the medulla of the brain of a minnow, 48 hr after infection, showing a larva in a blood vessel and other larvae near the blood vessel ventrally (*bl.*, blood vessel).

Fig. 6. Section through part of the brain of a minnow, 48 hr. after infection, showing a group of larvae around a blood vessel (*bl.*, blood vessel). Oral cap of spines visible in two larvae.

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NEW LARVAL MITES OF THE FAMILY TROMBICULIDAE (ACARINA: PROSTIGMATA)

By CHARLES D. RADFORD*

(With 22 Figures in the Text)

INTRODUCTION

In a collection of parasitic mites from Palestine sent by Prof. O. Theodor, Hebrew University, Jerusalem, the author found several larval mites which prove to be new species, one in the genus *Euschöngastia* the other two in the genus *Trombicula*. These are described and figured in the present paper along with a new species of the genus *Blankaartia* taken on a bird in Uganda and sent for identification by G. H. E. Hopkins, O.B.E. (formerly Senior Medical Entomologist, Kampala). The author wishes to acknowledge his thanks to the above donors for having given him the opportunity to examine these specimens.

In the case of *Euschöngastia spalaxia* sp.n., there is only the holotype, but there is a number of paratype slides of the two *Trombicula* species (*T. acomys* n.sp. and *T. palastinensis* n.sp.) and a distribution will be made later to as many laboratories as the material permits.

STANDARD DATA

AW—distance between antero-lateral setae; PW—distance between postero-lateral setae; SB—distance between sensory setal bases; ASB—distance from anterior of scutum to sensory bases; PSB—distance from posterior of scutum to sensory bases; A–P—distance between antero-laterals and postero-laterals; AM—length of antero-median seta; AL—length of antero-laterals; PL—length of postero-laterals; S—length of sensory setae; DS—length of dorsal setae; VS—length of ventral setae; pa—length of leg i; pm—length of leg ii; pp—length of leg iii.

Family Trombiculidae Ewing, 1944

Genus *Euschöngastia* Ewing, 1938

Euschöngastia spalaxia n.sp.

This species differs from all others of the genus by the shape of the dorsal scutum, the long postero-lateral setae and the ornamented sensory setae. It is nearest to *Euschöngastia aethomyia* (Radford, 1942), but differs from it by the shorter antero-lateral setae.

Dorsal scutum (Fig. 1) wider than long, the five scutal setae all placed on the anterior half of the scutum, the sensory setae are posterior to a line through the postero-laterals. The anterior edge of the scutum has a concavity on each side of

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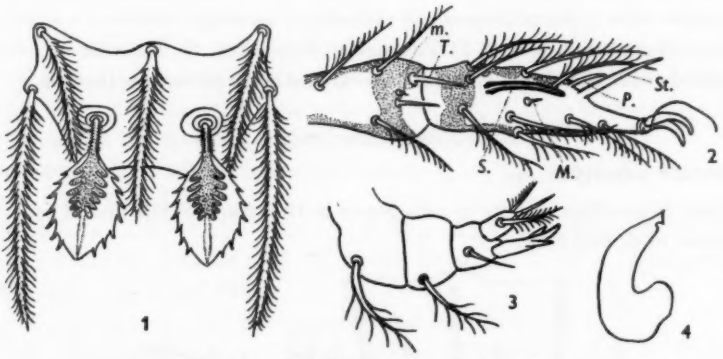
the antero-median seta; lateral edge of scutum concave; posterior edge of scutum directed backwards, with a central concavity. Scutum not flanked by eyes or eye-plates. Antero-median seta longer than the antero-laterals; postero-lateral setae about twice as long as the antero-laterals; all five scutal setae strongly feathered. The sensory setae are club-like, with serrated edges and with a punctate, leaf-like pattern.



Figs. 1–4. *Euschöngastia spalaxia* n.sp.

Fig. 1. Dorsal scutum. Fig. 2. Tarsus i. Fig. 3. Palp. *S.*, spur; *m.*, microspur; *St.*, subterminala; *P.*, parasubterminala; *M.*, microtibiala; *T.*, tibiala. Fig. 4. Chelicera.

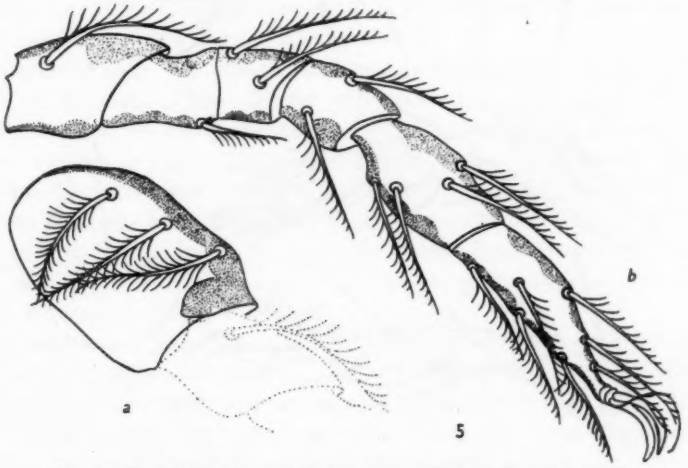


Fig. 5. *Euschöngastia spalaxia* n.sp. (*a*) Coxa iii. (*b*) Leg iii.

All legs with seven segments; tarsi with two claws and simple empodium. Coxa i and ii each bearing a single seta; coxa iii has three setae (Fig. 5*a*). Tarsus i (Fig. 2) with a long spur, microspur, subterminala and parasubterminala; tibia with 2 tibiala and a microtibiala. Leg iii (Fig. 5*b*) without mastitarsala.

Palpal femoral seta (Fig. 3) with lateral branches; genual seta with lateral branches; dorsal tibial seta nude; lateral tibial seta nude; ventral tibial seta with

lateral branches. Galeal seta with lateral branches. Palpal claw trifurcate. Chelicera (Fig. 4) with a single tooth.

Dorsal setae about 52; arranged in transverse rows 4, 4, 10, 2, 8, 8, 6, 6, 4. Ventral setae about 30; in rows 2, 2, 5, 5, 5, 5, 4, 2.

Standard data. AW, 73 μ; PW, 90 μ; SB, 33 μ; ASB, 29 μ; PSB, 16 μ; A–P, 20 μ; AM, 66 μ; AL, 60 μ; PL, 86 μ; S, 43 × 16 μ; DS, 53 μ; VS, 33/46 μ. Humerals, 73 μ; pa, 290 μ; pm, 283 μ; pp, 316 μ.

Host. A rodent (*Spalax kirgisorum ehrenbergi* Nehring, 1898).

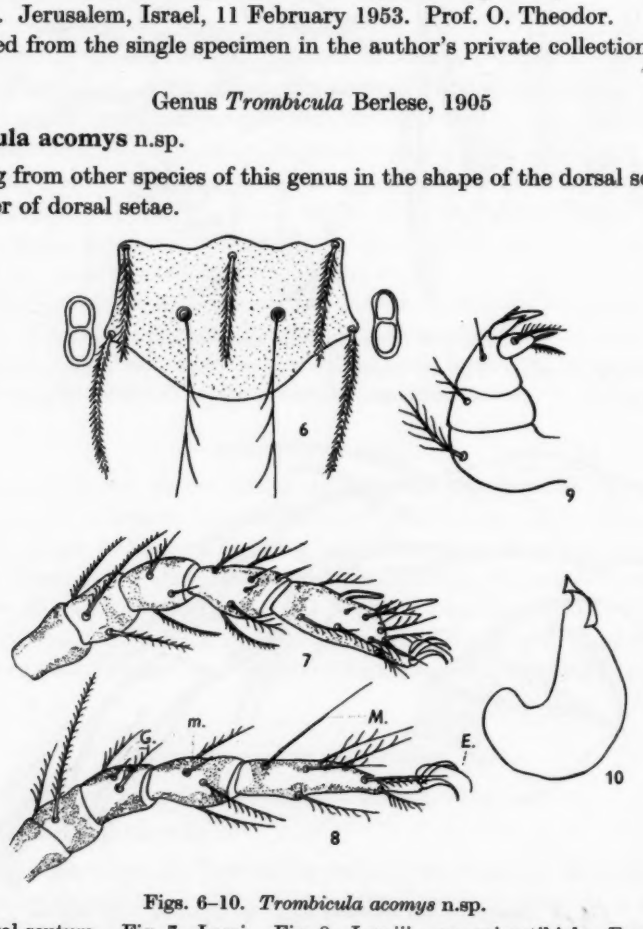
Locality. Jerusalem, Israel, 11 February 1953. Prof. O. Theodor.

Described from the single specimen in the author's private collection.

Genus *Trombicula* Berlese, 1905

Trombicula acomys n.sp.

Differing from other species of this genus in the shape of the dorsal scutum and the number of dorsal setae.



Figs. 6–10. *Trombicula acomys* n.sp.

Fig. 6. Dorsal scutum. Fig. 7. Leg i. Fig. 8. Leg iii. *m.*, microtibiala; *E.*, empodium; *M.*, mastitarsala. Fig. 9. Palp. Fig. 10. Chelicera.

Dorsal scutum (Fig. 6) broader than long, its surface punctate; anterior edge convex medially; lateral edges concave; posterior edge broadly convex. The antero-median seta is shorter than the other scutal setae and is placed some distance from the edge of the scutum. Postero-lateral setae longer than the

antero-laterals and placed farther apart. Sensory setae long, flagelliform, with some lateral branches. The sensory setal bases are placed midway between the anterior and posterior edges of the scutum. Paired eye-plates level with the postero-lateral setae, the posterior eye the larger.

All legs with seven segments; tarsi with two claws and an empodium. Coxae each with one seta. Leg i (Fig. 7) tarsus with a short, stout spur and a microspur, stout subterminala and a parasubterminala; tibia with 2 tibiala and a microtibiala; genu with a genuala. Leg iii (Fig. 8) with mastitarsala; tibia with tibiala; genu with genuala. Palp (Fig. 9) femoral seta with lateral branches; genual seta with lateral branches; dorsal tibial seta nude; lateral tibial seta nude; ventral tibial seta with lateral branches. Galeal seta nude. Palpal claw trifurcate. Chelicera (Fig. 10) wide at its base and with a tricuspid cap.

Dorsal setae about 46; in transverse rows 4, 8, 10, 8, 6, 4, 4, 2. Ventral setae 32; in rows 2, 2, 8, 4, 6, 6, 4.

Standard data. AW, 76 μ; PW, 90 μ; SB, 33 μ; ASB, 30 μ; PSB, 26 μ; A–P, 30 μ; AM, 40 μ; AL, 43 μ; PL, 56 μ; S, 76 μ; DS, 50 μ; VS, 30/43 μ. Humerals, 56 μ; pa, 256 μ; pm, 196 μ; pp, 260 μ.

Host. Desert rat (*Acomys cahirinus* Desmarest, 1819).

Locality. Mishmar Ha Emek, Israel, 28 November 1952. Prof. O. Theodor.

Type and paratypes in the author's private collection.

Trombicula palestinensis n.sp.

Differing from other species of the genus in the width of the dorsal scutum and the length of sensory and postero-lateral setae.

Dorsal scutum (Fig. 11) wider than long, its anterior edge being indented on each side of the median line; lateral edges diverging outwards to the postero-lateral corners; posterior edge broadly convex. Antero-lateral setae shorter than the antero-median, not placed in angles of the scutum; the antero-median seta is placed some distance below the level of the antero-laterals; postero-lateral setae wider apart than the antero-laterals and about twice their length; sensory setal bases level with a line drawn through the postero-laterals; sensory setae long, flagelliform, with lateral branches. Paired eye-plates flanking the postero-lateral setae, the anterior eye being the larger.

All legs with seven segments; tarsi with two claws and a slender empodium; all coxae with a single seta. Tarsus i (Fig. 12) with a slender spur, subterminala and parasubterminala; tibia with two tibiala and a microtibiala; genu with three genuala. Tarsus ii (Fig. 13) with a spur and microspur; tibia with two tibiala; genu with a genuala. Tarsus iii (Fig. 14) with a long mastitarsala; tibia with a short tibiala; genu with a genuala.

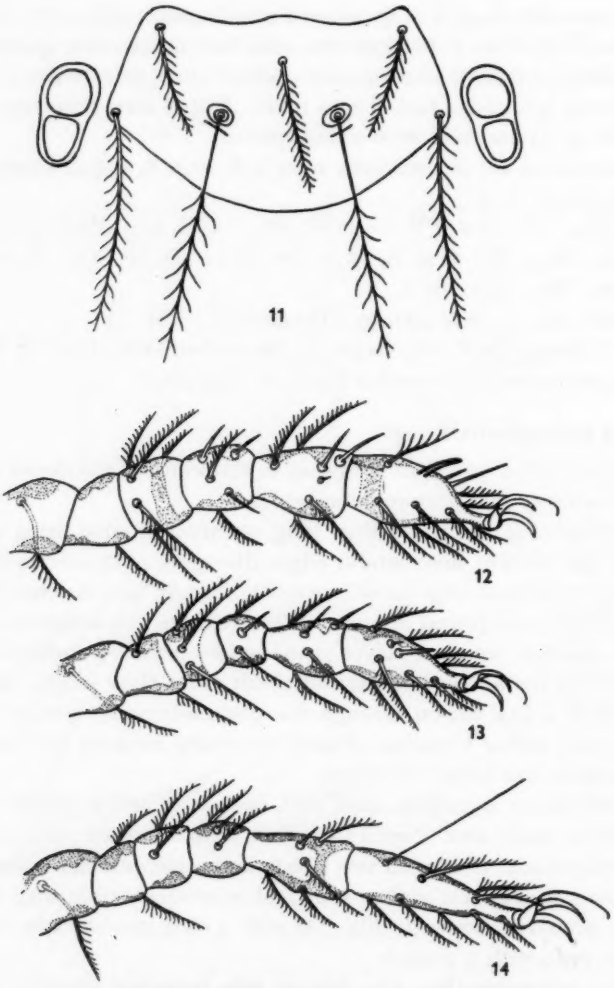
Palpal claw tridentate (Fig. 15); femoral seta branched laterally; genual seta branched; dorsal tibial seta long, simple, nude; lateral tibial seta nude; ventral tibial seta branched. Galeal seta nude. Chelicera (Fig. 16) with a tricuspid cap.

Dorsal setae about 52; arranged in transverse rows 4, 8, 10, 10, 8, 6, 4, 2. Ventral setae 42; in rows 2, 2, 8, 2, 6, 6, 6, 4, 4, 2.

Standard data. AW, 70 μ; PW, 93 μ; SB, 33 μ; ASB, 33 μ; PSB, 20 μ; A–P, 30 μ; AM, 43 μ; AL, 40 μ; PL, 56 μ; S, 20 μ; DS, 13 μ; VS, 30/40 μ. Humerals, 56 μ; pa, 243 μ; pm, 223 μ; pp, 266 μ.

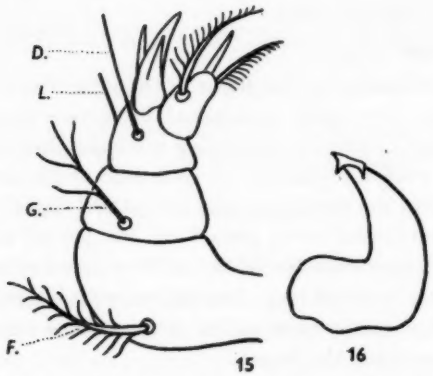
Host. Desert rat (*Acomys cahirinus* Desmarest, 1819).

Locality. Mishmar Ha Emek, Israel, 28 November 1952. Prof. O. Theodor. Type and paratypes in the author's private collection.

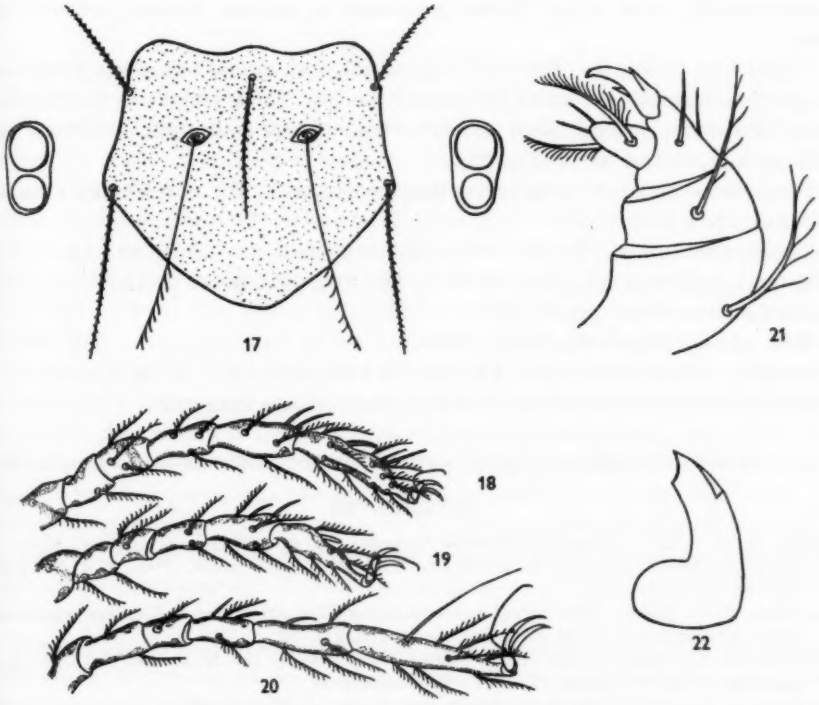


Figs. 11–14. *Trombicula palestinensis* n.sp.

Fig. 11. Dorsal scutum. Fig. 12. Leg i. Fig. 13. Leg ii. Fig. 14. Leg iii.



Figs. 15, 16. *Trombicula palestinensis* n.sp.
Fig. 15. Palp. Fig. 16. Chelicera.



Figs. 17–22. *Blankaartia laniarius* n.sp.
Fig. 17. Dorsal scutum. Fig. 18. Leg i. Fig. 19. Leg ii. Fig. 20. Leg iii.
Fig. 21. Palp. Fig. 22. Chelicera.

Genus *Blankaartia* Oudemans, 1911

Syn. *Tragardhula* Berlese, 1912

Blankaartia laniarius n.sp.

Differing from other species of the genus in the large size of the dorsal scutum.

Dorsal scutum (Fig. 17) roughly pentagonal, its surface being finely but densely punctate; anterior edge of scutum protruding some distance above the level of the antero-lateral setae, with a concavity on each side of the median line. Antero-median seta longer than the antero-laterals and placed slightly above a line drawn through these; antero-lateral setae placed on the lateral edge of the scutum; postero-lateral setae longer than the others; all five scutal setae are furnished with only short barbs. Sensory setae long, flagelliform with lateral barbs on the inner, distal third. Paired eye-plates flanking the scutum, level with the postero-lateral setae, the anterior eye being the larger.

All legs with seven segments; tarsi with two claws and a slender empodium. Leg i (Fig. 18) tarsus with a short, stout spur; long, slender subterminala and parasubterminala; tibia with two tibiala; genu with a genuala. Leg ii (Fig. 19) tarsus with a short, stout spur; tibia with two tibiala. Leg iii (Fig. 20) tarsus with a mastitarsala; tibia with a tibiala; genu with a genuala. Coxae, each with one seta.

Palpal claw tridentate (Fig. 21); femoral seta with lateral branches; genual seta longer than femoral seta and with lateral branches; dorsal tibial seta nude; lateral tibial seta nude; ventral tibial seta branched. Galeal seta nude. Chelicera with a dorsal and ventral tooth (Fig. 22).

Dorsal setae about 26; arranged in transverse rows 2, 6, 6, 8, 4. Ventral setae 18; in rows 2, 2, 4, 2, 2, 2, 4.

Standard data. AW, 93 μ; PW, 96 μ; SB, 40 μ; ASB, 33 μ; PSB, 56 μ; A–P, 33 μ; AM, 50 μ; AL, 40 μ; PL, 66 μ; S, 86 μ; DS, 66 μ; VS, 50/66 μ. Humerals, 83 μ; pa, 360 μ; pm, 343 μ; pp, 410 μ.

Host. A bird (*Laniarius erythrogaster*).

Locality. River Otiro, Hulu, Uganda, 24 December 1937. T. W. Chorley.

The type and two paratypes in the author's private collection.

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RHIPICEPHALUS HUMERALIS RONDELLI 1926

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(With 7 Figures in the Text)

INTRODUCTION

Rhipicephalus humeralis was first described by Rondelli in 1926 from specimens collected from an unknown host at Mogadiscio, Somalia. Only the male was described, the female and immature stages being unknown at that time. The following descriptions of the male, female, nymph and larva are made from specimens bred in the laboratory, the progeny of a female collected from an elephant at Mackinnon Road (approximately fifty miles north-west of Mombasa, Kenya) on 3 October 1951 by D. L. W. Sheldrick.

DESCRIPTION

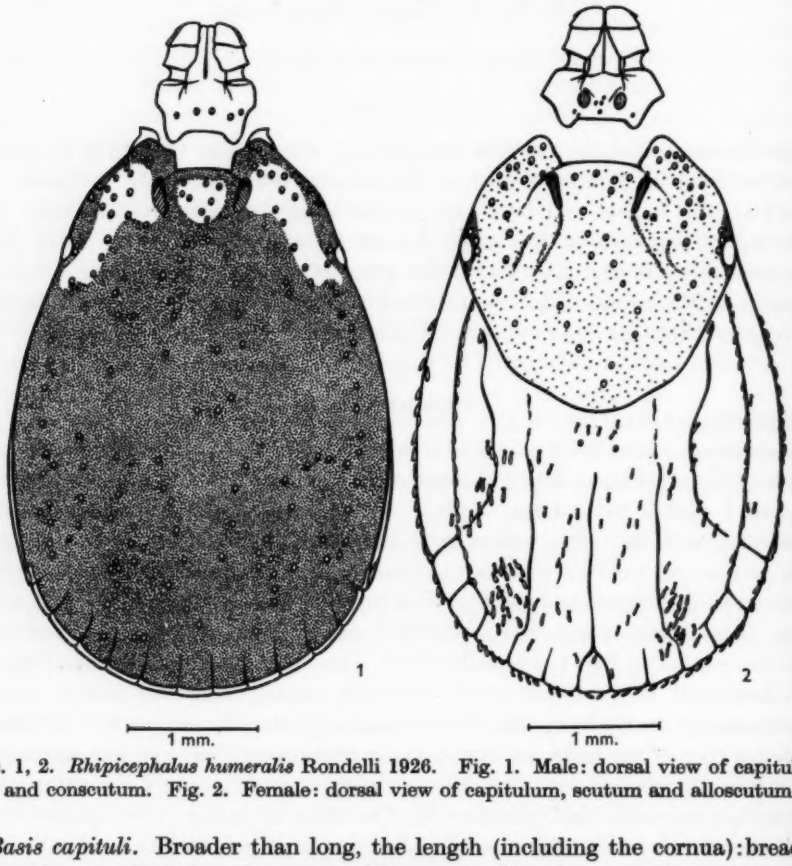
Male (Figs. 1, 7).

Conscutum. Size 4·5 × 3 to 2·8 × 1·9 mm. Oval in shape with widest part over half-way back. Ornate, colour pattern often not very striking. Anterior process of coxa I visible from dorsal surface. Emargination narrow and deep. Eyes relatively small, flat, often edged by a few punctations dorsally, otherwise flush with conscutum. Cervical pits deep and convergent. No cervical, lateral, marginal, postero-median or postero-lateral grooves present. Festoons well-developed, sometimes more or less even in size, sometimes central festoons are slightly narrower than the rest, as in Fig. 1, Rondelli (1926). Caudal process, with terminal plaque, well-developed in specimens which have fed, arising from the central festoon. In such specimens the body wall tends to expand postero-laterally, thus making the posterior part of the body, excluding the caudal process, more or less rectangular in outline. Punctation pattern made up of discrete medium-sized punctations with numerous small interstitial punctations. The latter are always very fine and superficial, sometimes practically obsolete. Medium-sized punctations tend to be concentrated on the scapulae, in lines along the positions of the marginal grooves and in the positions of the postero-lateral grooves, elsewhere on the conscutum they are scattered haphazardly. Foveae very indistinct in some specimens, easily seen in others.

The conscutum is predominantly very dark brown in colour with a pattern of light smoky-brown enamelled patches anteriorly. A light patch always appears to be present on each scapula and often between the cervical pits. In addition, there may be an isolated patch behind each eye; in some cases these patches behind the eyes extend forwards and join those on the scapulae, as in Fig. 1. This figure illustrates the maximum development of the enamelled markings on the conscutum seen to date in specimens of this species.

Legs. Increase in size from I to IV. Mottled white enamelling is present on the dorsal surface of the legs, particularly on III and IV.

Capitulum. Longer than broad, the proportions usually being 5:4. Dark brown in colour, sometimes slightly enamelled on the basis capituli just posterior to the palps. Subcollare present.



Figs. 1, 2. *Rhipicephalus humeralis* Rondelli 1926. Fig. 1. Male: dorsal view of capitulum and conscutum. Fig. 2. Female: dorsal view of capitulum, scutum and alloscutum.

Basis capituli. Broader than long, the length (including the cornua):breadth proportion usually being about 3:4. Antero-lateral borders straight and directed almost straight back, postero-lateral borders longer, concave and convergent posteriorly. The lateral angles are, therefore, obtuse and far forward. Posterior border concave to straight with strong cornua. A row of punctations is present roughly parallel with the posterior border.

Palps. Article I easily visible from the dorsal surface. Article II longer than article III; the latter tapers slightly towards the tip.

Ventral surface. Anal plate (Fig. 7). Relatively long and narrow, being over twice as long as broad. External margin long and straight, joining the slightly convex posterior margin in a smooth curve. Internal margin sinuous, joining the posterior margin in a right angle. *Accessory anal plates* absent.

Female (Fig. 2). Size 4·3 × 2·9 to 3·2 × 2·2 mm.

Scutum. Ornate. Somewhat variable in form, usually as broad as long but in some cases slightly longer than broad, in others slightly broader than long; broadest at eye level, about half-way back. Posterior margins slightly sinuous. Emargination wide and deep. Eyes slightly convex, with a few punctations along the dorsal edge. Long convergent cervical pits present but no true cervical or lateral grooves. Sometimes there are slight, rather smoky-coloured depressions in the position of the cervical grooves, as indicated by the stippling in Fig. 2. As in the male the punctation pattern is made up of discrete medium-sized punctations interspersed with small punctations. The latter are numerous, sometimes well defined, at other times virtually obsolete. Medium-sized punctations are grouped on the scapulae and often form rather ill-defined lines in the position of the lateral grooves. Elsewhere they are scattered.

The scutum is predominantly slightly brownish-cream in colour with darker smoky-brown colouring round the eyes. Often this darker coloration extends forwards along the edges of the scutum and sometimes round the tips of the scapulae and between the cervical pits. This coloration, however, never appears to be very dark.

Alloscutum. Dark brown in colour and bearing a number of white clavate hairs, though these are not nearly so numerous as they are in the female of *R. pulchellus*.

Legs. Mottled white enamelling present on the backs of the legs, as in the male.

Capitulum. Slightly longer than broad. Brown in colour with slight enamelling on the basis capituli posterior to the palps. Small subcollare sometimes present.

Basis capituli. Twice as broad as long. Antero-lateral borders straight, postero-lateral borders concave, lateral angles acute, approximately half-way back. Posterior margin straight and cornua strong. Porose areas oval, wide apart. The central part of the basis capituli enclosing the porose areas is usually sunken.

Palps. Article I easily visible from dorsal surface. Article II longer than article III; the latter has a bluntly rounded tip.

Nymph (Figs. 3–4).

Scutum. Broader than long, broadest half-way back at eye level. Antero-lateral margins convex, postero-lateral margins slightly sinuous, smoothly rounded at the end. Emargination wide and shallow. Eyes large. Cervical pits deep; cervical fields sunken and demarcated by cervical and lateral grooves. Cervical grooves deep and convergent at first, becoming shallower and divergent and fading out at about the level of the anterior limit of the eyes. Lateral grooves long, running almost parallel to the sides of the scutum for most of their length then diverging slightly and ending just inside the postero-lateral margins of the scutum.

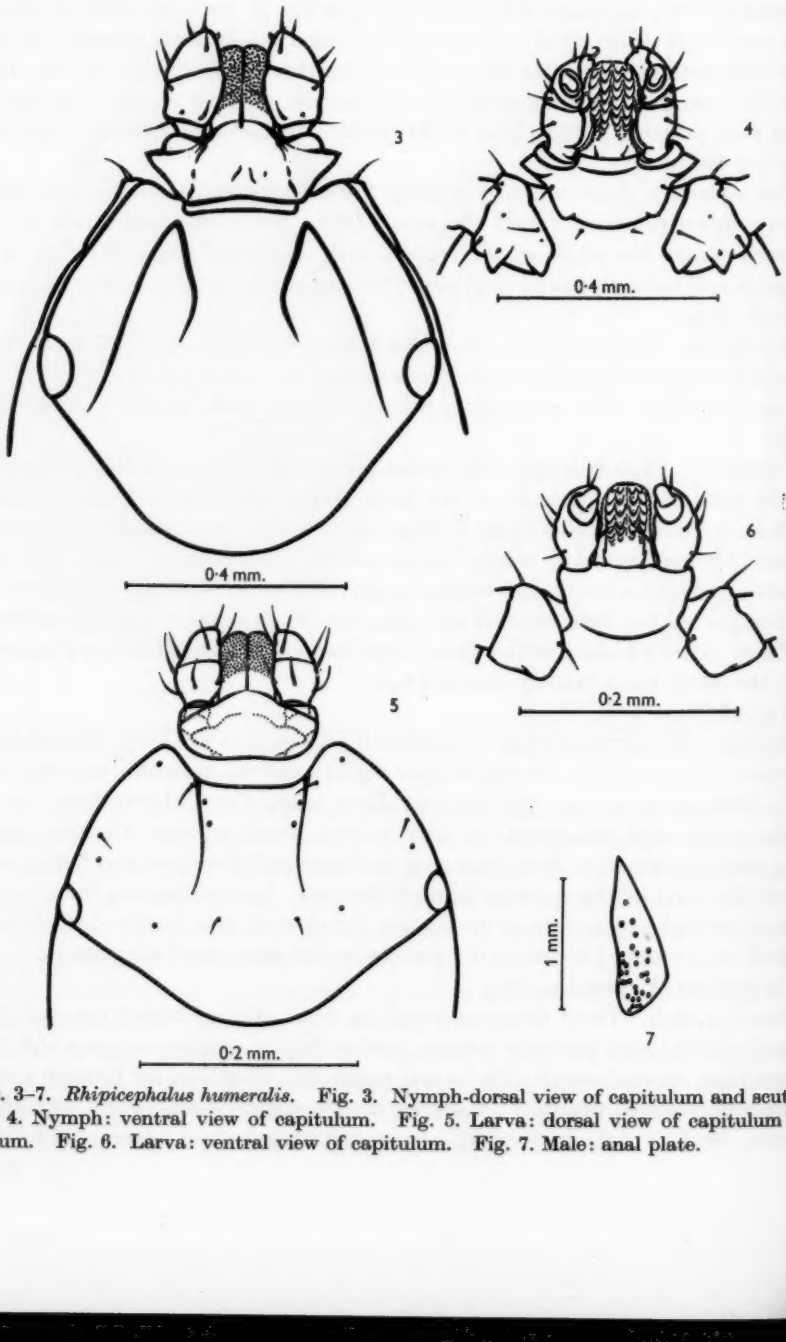
Capitulum. As broad as long.

Basis capituli. Three times as broad as long. Antero-lateral margins short, almost parallel with posterior margin, postero-lateral margins concave and much longer than antero-laterals. The lateral angles are, therefore, far forward and are sharp and recurved ventrally. Posterior margin slightly concave and virtually no cornua. Surface of the basis capituli somewhat irregular and interrupted by slight

grooves and ridges. Ventrally spurs are present at the junction of the concave postero-lateral margins with the convex posterior margin.

Palps. Normally overlap the hypostome. Article I easily visible dorsally, article II longer than article III; the latter tapers to a bluntly rounded tip. Ventrally a retrograde spur is present on article III.

Larva (Figs. 5, 6).



Figs. 3–7. *Rhipicephalus humeralis.* Fig. 3. Nymph-dorsal view of capitulum and scutum. Fig. 4. Nymph: ventral view of capitulum. Fig. 5. Larva: dorsal view of capitulum and scutum. Fig. 6. Larva: ventral view of capitulum. Fig. 7. Male: anal plate.

Scutum. Much broader than long, broadest at eye level, just over half-way back. Antero-lateral margins convex, postero-lateral margins very slightly sinuous, smoothly curved at the end. Emargination wide and deep. Eyes large. Cervical grooves extend backwards to eye level, slightly convergent at first, becoming slightly divergent farther back.

Capitulum. About as broad as long.

Basis capituli. Twice as broad as long. Almost right-angled antero-laterally, very slightly convex laterally and curving smoothly postero-laterally to join the slightly concave posterior margin. No spurs ventrally.

Palps. Article I just visible dorsally, article II slightly longer than article III, which tapers slightly to a blunt end.

Holotype. Male from an unknown host, Mogadiscio, Somalia, Rondelli 1926. Deposited in the collection of the Museum of Natural History, University of Turin, under the name of *Rhipicephalus pulchellus humeralis*.

Males, females, nymphae and larvae from the laboratory-bred series, reference number B.S. 274/–, East African Veterinary Research Organization collection, on which the above descriptions are based, have been deposited in the collections of the British Museum (Natural History); Museum of Natural History, University of Turin; the Veterinary Research Laboratory, Onderstepoort and H. Hoogstraal.

DISCUSSION

R. humeralis was originally described by Rondelli as a subspecies of *R. pulchellus*; it was also considered as such by Zumpt (1942). Zumpt (1949) raised it to specific status.

The author considers it unlikely that the males of *R. humeralis* and *R. pulchellus* have been confused in the past. Warburton (1932) stated that he had received a number of ticks from Somalia, identified as a variety of *R. pulchellus*, 'in which all the white markings were obsolete except patches on the scapulae'. These, obviously, were specimens of *R. humeralis*. He added that 'the same consignment contained intermediate forms clearly linking these up with full patterned specimens' and suggested that there was a possibility that the two forms might have been confounded when the white pattern of *R. pulchellus* was largely obsolete. A comparison of laboratory-bred series of *R. humeralis* and *R. pulchellus* does not support this suggestion since no intermediate forms have been seen. Cunliffe (1913) illustrated an exceptionally dark specimen of *R. pulchellus*, but no specimen of *R. humeralis* seen by the author approaches the coloration of this form any closer than does the one shown in Fig. 1.

Rondelli (1926) pointed out that three males from Somalia identified by Paoli (1916) as *R. ecinctus* (a species which was subsequently established by Warburton (1912) as a synonym of *R. maculatus*) were in fact specimens of *R. humeralis*.

It is, however, difficult to distinguish between the females of *R. humeralis* and *R. pulchellus*, and it is very probable that they have been confused in the past. A careful comparison of laboratory-bred specimens reveals that there are a number of differences which should serve to separate the two species. The coloration of the

scutum of *R. humeralis* is predominantly brownish-cream, with darker smoky-brown ornamentation, compared with the ivory and the dark grey to black coloration of *R. pulchellus*. Similarly, the alloscutum of *R. humeralis* is dark brown, while that of *R. pulchellus* is blackish-brown. These differences in colour are seen both in living specimens and, even more markedly, in specimens preserved in 5% formalin saturated with chloroform. The punctations on the scutum of *R. humeralis*, both medium-sized and fine, are often somewhat larger and deeper than those of *R. pulchellus*, hence the scutum is not nearly so smooth and flat in appearance as it is in the latter species. The clavate hairs on the alloscutum, characteristic of both species, are neither so large nor so numerous in *R. humeralis* as they are in *R. pulchellus*. Finally, the degree of mottled enamelling on the dorsal surface of the legs of both species is not so marked in *R. humeralis* as it is in *R. pulchellus*.

It is also difficult to differentiate the nymphae of these two species. The principal difference between them lies in the shape of the basis capituli. In *R. humeralis* it is broader than it is in *R. pulchellus*, and the lateral angles point sideways and do not sweep forwards as they do in *R. pulchellus*. These differences can be seen in both dorsal and ventral views of the ticks.

It is virtually impossible to distinguish the larvae of *R. humeralis* and *R. pulchellus* from each other.

Laboratory rearing

R. humeralis is a three-host tick, easy to rear in the laboratory.

Egg laying and hatching took place in an incubator at 25–27° C. and approximately 70% relative humidity, and the larvae were then transferred to a cupboard at room temperature, which fluctuated between 16 and 23° C, for storage until they were fed. Similarly, engorged larvae and nymphae were placed in the incubator to moult and were then transferred to the cupboard until they were fed again. All stages fed well on the ears of rabbits. Accurate records of the periods required by larvae, nymphae and adults for hardening were not kept but the times required for other phases of development were as follows: Preoviposition period 8–11 days at 25–27° C. and approx. 70% R.H.; larvae hatch in 35–40 days at 25–27° C. and approx. 70% R.H.; larvae engorge in 3–5 days; larvae moult in 12–17 days at 25–27° C. and approx. 70% R.H.; nymphae engorge in 3–7 days; nymphae moult in 16–25 days at 25–27° C. and approx. 70% R.H.; adults engorge in 9–15 days.

Survival periods

No accurate data are available.

Hosts and distribution

As yet, host and distribution records for *R. humeralis* are relatively few and far between. It has only been recorded from Somalia, Kenya and Tanganyika (Table 1).

These records show that *R. humeralis* has a preference for hot, low-lying country with an average annual rainfall of 20–30 in. or less. Glover (personal communication) states that the vegetation over large areas of Somalia is dry *Acacia-Commi-*

phora scrub. This type of scrub vegetation is also found in the areas in Kenya in which the tick is most common, i.e. between Kiboko and Mackinnon Road, north-west of Mombasa. Manyoni, in Tanganyika, is in dry Itigi thicket country.

Disease transmission

Has yet to be investigated.

Table 1

Synonym	Hosts	Locality	Collector	Authority
		Somalia		
R. ecinctus	—	Bassa Goscia	—	Paoli (1916)
R. ecinctus	Mule	Matagoi	—	Paoli (1916)
R. ecinctus	Camel	Jac Doudou	—	Paoli (1916)
R. pulchellus humeralis	—	Mogadiscio	Romualdo Ganora	Rondelli (1926)
		Kenya		
—	Cattle, sheep, camel, elephant, rhino, eland, hartebeest, bush-buck	Most common between Kiboko and Mackinnon Road, including the southern parts of Ukamba. Also occurs, but is less abundant, at Isiolo, on the North Yatta plateau and north of Malindi	—	Wiley (1955, personal communication)
—	Elephant	Mackinnon Road	D. L. W. Sheldrick	Author
		Tanganyika		
—	Rhino	Manyoni district	S. M. Moore-Gilbert	Author

SUMMARY

1. The male of *Rhipicephalus humeralis* is redescribed and the female and immature stages are described for the first time. The synonymy of the species is discussed and the various stages are compared with those of *R. pulchellus*.
2. Details of the life cycle under laboratory conditions are given.
3. The host list and distribution of the species are given.

The author wishes to thank the Director, East African Veterinary Research Organization, for permission to publish this paper, and the following workers for their generous assistance: D. L. W. Sheldrick, Warden of the Royal Tsavo National Park (East), Voi, for collecting living specimens of *R. humeralis*; Dr G. Theiler, Onderstepoort, H. Hoogstraal, U.S. N.A.M.R.U. 3, Cairo, and Dr S. F. Barnett, East African Veterinary Research Organization, for checking the manuscript; Dr P. E. Glover, Veterinary Research Laboratory, Kabete, for information on the vegetation of the areas in which this tick occurs and A. J. Wiley, Veterinary Research Laboratory, Kabete, for information on the distribution of the tick in Kenya and permission to quote unpublished data.

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THE STUDY OF THE TRANSMISSION OF THE CATTLE LUNGWORM (*DICTYOCAULUS VIVIPARUS*) IN RELATION TO PASTURE CONDITIONS

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(With Plates XII and XIII)

INTRODUCTION

The number of lungworm larvae ingested by the grazing animal has a considerable bearing on the severity of the resulting infection. There is an increasing volume of evidence (Michel & Shand, 1955) to suggest that these numbers depend more upon the nature of the herbage and the consistency of the faeces than on the numbers of larvae being passed on to the ground in the faeces.

An investigation of the factors affecting the transmission of lungworm from one animal to another must therefore include a study of the role of the pasture. The number of larvae available to the grazing animal is a complex concept which depends not only on the development of the larvae to the infective stage, their survival and their position on the herbage (Michel & Rose, 1954), but also on the grazing behaviour of the animal (Taylor, 1954; Michel, 1955).

Each of these factors is influenced by the pasture, by the nature of the sward, or indirectly, by the micro-climate associated with it. The process of transmission is affected by many such factors, but it is neither necessary nor desirable to study them in isolation.

What is required is some understanding of the process as a complex of inter-related factors. Allied to this is the problem of how to measure the process in the field.

The present series of experiments was designed to investigate one specific problem: the effect of length of herbage on transmission. Basically, the comparison was between long and short swards, but the latter were produced in three ways. There were, therefore, four treatments: (1) grazed by resistant cattle; (2) grazed by sheep; (3) gangmown; (4) long.

EXPERIMENTAL METHODS

The experimental plan was to graze each paddock with two experimentally infected calves (passing larvae in their faeces) for 1–2 weeks and, a fortnight after their removal, to graze each plot with two calves raised free from lungworms.

Six such experiments were carried out over a 2-year period (June 1954–June 1956); only the first year's work (Expts. 1–3) is reported here. The dates when grazing by infected and test calves took place are given in Table 1.

The result of each experiment was assessed by the post-mortem examination of the test calves and the determination of their worm burden, and also by the estimation of the concentration of larvae on the herbage; the latter was carried out by a modification of Taylor's (1939) technique, as described by Michel & Parfitt (1955). These determinations were carried out weekly from the time the plots were infected and normally continued until several successive examinations proved negative. This enabled the decline in the larval population on the grass to be followed.

Expts. 1 and 2 were carried out at Drayton, Stratford-on-Avon, on a second-year ryegrass/white clover ley. This was a dense sward with a very high proportion of clover, as may be seen from Pl. XII, figs. 1 and 2 (long and gangmown respectively).

Expt. 3 was carried out at Hurley, Berks, on an outrun ley with a dense growth of herbage near the soil surface but a very open growth above it (Pl. XIII, figs. 3, 4). There was, therefore, a much greater difference between treatments in the first two experiments than in the third.

Table 1

	Date of grazing of plots			
	Infecting calves		Test calves	
Expt.	On	Off	On	Off
1	30. vi. 54	13. vii. 54	28. vii. 54	4. viii. 54
2	30. ix. 54	14. x. 54	28. x. 54	4. xi. 54
3	25. iv. 55	9. v. 55	24. v. 55	31. v. 55

Treatments 1–3 were imposed as and when required to maintain a short grass sward, and a special point was made of imposing them at least once between the time when the infecting calves were placed on each plot and the time when the test calves went on. Treatment 4 involved cutting at some time before the experiment, after which the grass was allowed to grow undisturbed. This was intended to produce long herbage (over 4 in. in height) but, during conditions not conducive to growth, sometimes resulted in a shorter sward than was thought desirable.

The calves used to infect the paddocks were raised and artificially infected at Weybridge. Regular faecal samples were taken and the numbers of larvae estimated in 10 g., as described by Parfitt (1955).

During the period when the infecting calves were on the plots, faecal examinations were carried out on each calf every other day. There was considerable variation in the faecal larval count of the infecting calves and it was not possible, using two calves to infect each plot, to ensure that an equivalent number of larvae were passed on to each paddock. In order to avoid large differences the pairs of calves used were balanced as far as possible.

Differential infections, nevertheless, occurred within each experiment. The methods used to calculate and express the results, described in the following section, compensated for these smaller differences.

The test calves were reared indoors either at Drayton or Hurley under conditions precluding infection with lungworms. They were placed on the plots when they

were approximately 3 months old and remained there for one week. They were then maintained indoors for a further fortnight, to allow any larvae ingested to reach the lungs, and then slaughtered at Weybridge.

The mean faecal larval counts for the infecting calves were calculated for the period when the calves were on the plots. They were based on the curve of numbers of larvae found in the faeces over this period. Similarly, the mean grass counts refer to the period during which the test calves were on the plots and were calculated from the curve of decrease of larval numbers on the herbage during this period.

RESULTS AND DISCUSSION

The results of the post-mortem examinations are tabulated (Table 2); the data derived from the estimations of numbers of larvae on the grass are given in Table 3.

Table 2. *Post-mortem data on test calves. Total numbers of* Dictyocaulus viviparus

Expt.	Calf no.	Plot	Total count	Mean count
1	68,221	1	2987	3540
	68,218		4092	
	68,220	2	5761	5985
	68,223		6209	
	68,224	3	4347	3202
	68,226		2058	
	68,227	4	2422	2040
	68,222		1657	
2	47,215	1	1257	1169
	48,268		1081	
	47,218	2	850	1049
	47,220		1248	
	47,214	3	1085	2612
	47,219		4139	
	48,267	4	2303	5640
	47,216		8977	
3	B79	1	734	593
	3637		452	
	B74	2	24	272
	3647		520	
	B73	3	973	813
	3638		653	
	B77	4	225	252
	3648		278	

In Expt. 1, during the summer of 1954, the number of larvae per lb. of herbage rose rapidly to a peak on all plots and declined to zero 8 weeks after the infecting calves were removed (Table 3). In the autumn (Expt. 2) the counts persisted at a low level for a further month. In the following spring (Expt. 3) counts declined to zero in 6 weeks. It is of interest that on Paddock 2 larvae were detected on the grass only once (1·8 per lb. on 17 May 1955) and the count was zero when the test calves were on it. Yet the test calves acquired considerable numbers of worms.

The evaluation of the influence of pasture conditions on the transmission of lungworm infection is in part a problem of methodology, and it is essential to

express the results in such a way that treatments may validly be compared. To achieve this the data have been expressed as ratios. A ratio of this type was used by Michel & Mackenzie (1956) to represent the proportion of the larvae being passed in the faeces of the infecting calves which were found in the lungs of the test calves. This ratio has been referred to as a 'transmission' coefficient, since it is concerned with the total 'calf-to-calf' process of transmission of infection.

Any variation in the time elapsing between grazing by infecting and test calves necessarily affects the process of transmission, by affecting the survival of larvae on the pasture. Such coefficients, therefore, have no absolute value but, within an experiment, have a relative value. Thus, in each of the present experiments a high transmission coefficient indicated conditions conducive to the production of disease.

Table 3. *Grass counts. Larvae per lb. herbage*

	Expt. 1 (summer). Week 1 = 6. vii. 54				Expt. 2 (autumn). Week 1 = 6. x. 54				Expt. 3 (spring). Week 1 = 10. v. 55			
Plot ...	1	2	3	4	1	2	3	4	1	2	3	4
Week 1	0	0	0	0	0	0	0	0	19·9	0	12·1	13·5
2	1	86·7	27	91·8	1·1	2	15	14	7·8	1·8	26·4	11·1
3	17	82·7	124·1	76·2	7·4	25·6	6·9	83·4	0	0	19	1·3
4	21	38·8	28·1	24·2	20	10	11	87·5	1·6	0	3·8	4
5	0	0	16	0	3·2	1	39	27·3	0	0	3·7	0
6	2	10	2·1	2	2	0	3	5·1	0	0	0	1
7	0	3·1	3	0	1·8	3·1	4·1	42	0	0	0	0
8	0	0	0	0	1	0	0	37·8	0	0	0	0
9	0	0	0	0	3	2·1	0	0	0	0	0	0
10	0	0	0	0	0	2	0	1	—	—	—	—
11	0	0	0	0	0	0	0	2	—	—	—	—
12	—	—	—	—	0	0	3	0	—	—	—	—
13	—	—	—	—	0	0	2	1	—	—	—	—

The transmission process may be considered in two main parts, and these in turn may be expressed as coefficients. A 'Translation' coefficient may be used to express the proportion of larvae in the faeces of the infecting calves which are detectable on the grass. An 'Acquisition' coefficient may be calculated as the proportion of larvae on the grass which succeed in establishing themselves in the lungs of the test calves. The mean counts for the first three experiments, of larvae in the faeces and on the grass and of worms in the lungs, are given in Table 4, together with the calculated coefficients.

The results of this first year are of value chiefly in so far as they elucidate methodological problems: their value as a contribution to knowledge of how lungworm infection is transmitted under different pasture conditions cannot be fully assessed until they are compared with the results of the second year's work.

Tentative conclusions may be drawn about both these aspects. As a method of study the use of the three coefficients, as defined above, offers considerable promise. The value of these coefficients, however, depends largely on the validity of the measurements upon which they are based. Clearly, the number of larvae per unit of faeces does not necessarily reflect the total number of larvae actually being

passed on to the pasture. The total faecal output and its dry-matter content affect this relationship but, in so far as they both differ between treatments, they are consequences of the treatments. Thus, loose faeces due to pasture conditions may lower the larval count per unit of faeces. The nature of the faeces may, in turn, lead to a greater distribution of faecal matter and infection, giving rise to a high grass count. The resulting (higher) translation coefficient will then reflect this complex pattern and indicate pasture conditions conducive to part of the transmission process.

Similarly, the grass count per lb. of herbage will depend partly on the volume of herbage present on the plot and the manner in which the infected faeces are spread on it, and the result will be affected by the selective grazing behaviour of the test animal. All these are influenced by, and are to some extent products of the pasture conditions.

Table 4

Plot no.	Mean infecting calf faecal larvae count A	Mean grass count B	Mean test calf worm count C	Transmission C/A	Translation B/A	Acquisition C/B
			Expt. 1. Summer 1954			
1	387	14·9	3540	9·15	0·0385	237·6
2	698	27·1	5985	8·58	0·0388	220·8
3	196	19·7	3203	16·4	0·1005	164·1
4	191	15	2040	10·7	0·0786	136·1
			Expt. 2. Autumn 1954			
1	1600	7·4	1169	0·731	0·00463	158·1
2	1980	3·2	1049	0·53	0·00162	327·8
3	416	30·4	2612	6·48	0·0731	85·9
4	3865	41·8	5605	1·45	0·0108	110·8
			Expt. 3. Spring 1955			
1	410·4	0·4	593	1·446	0·00098	1485
2	66·3	0	272	4·10	—	—
3	667·6	7·03	813	1·22	0·015	116·0
4	406·4	1·14	252	0·62	0·0029	221·2

The acquisition coefficient, therefore, reflects this complex pattern *because* it is based on the larval count per lb. of herbage.

This method of expressing the results enables a comparison to be made of the process of transmission under a variety of pasture and climatic conditions. In the first three experiments the weather was generally wet and it was not possible to study the process under hot, dry conditions.

The general approach, however, appears to be applicable to the study of the part played by climatic as well as pasture conditions.

An examination of Table 2 reveals the wide variation that is possible between the post-mortem worm counts of a pair of calves on the same plot, exposed to the same pasture infection. This suggests that experiments of this type should be carried out with larger numbers of animals than were used here. The use of too many calves, however, may tend to alter the pasture conditions under investigation.

Reference to Table 4 shows that, in Expts. 1 and 2, treatment 3 produced a transmission coefficient of a markedly higher order than that of the other three treatments. The two grazing treatments (1 and 2) were much lower and the long grass (4) gave an intermediate figure. In Expt. 3 the results were quite different, perhaps because of the generally shorter grass and consequently smaller differences between treatments which obtained in this experiment.

The translation process paralleled that of transmission in the summer and autumn but not in the spring. It is noticeable, however, that the translation coefficients for the different treatments follow the same pattern in each experiment (Table 4). This suggests that the gangmowing treatment was conducive to the process of translation. It has been suggested that only very small numbers of larvae succeed in migrating on to the grass from undisturbed faeces (Michel & Rose, 1954). Mechanical spreading of faeces, as by gangmowing, would therefore tend to spread infection and increase translation. The practical significance of this would depend upon the subsequent survival of the larvae, which would be greatly influenced by temperature and humidity.

It would seem that in the summer and autumn translation was the major factor in the total process of transmission. In the spring the acquisition phase appeared to be more important.

There are two consistent features in the results from all three experiments. One has already been mentioned, the tendency for the translation coefficients to follow a similar pattern in each experiment. A similar consistency may be observed for the acquisition coefficients. A comparison of the two coefficients, however, shows a remarkably uniform tendency for the acquisition coefficients to be in inverse order to those for translation. Thus although the order of transmission coefficients was not consistent in all three experiments, there was evidence of consistency in the order of both translation and acquisition coefficients.

The total process may be regarded as a combined effect of translation and acquisition. There appears to be no theoretical reason why the two processes should not, at times, work in the same direction. Yet it appears that, in the experiments described, high translation was associated with low acquisition. Thus the latter appeared to modify the former and, to this extent, impaired the relationship between grass count and post-mortem worm count.

It nevertheless remains true that the numbers of larvae actually on the grass were much more important than the numbers being passed on to it, as a guide to the severity of infection produced in the test calves.

SUMMARY

1. A series of experiments is described in which the transmission of cattle lungworm infection has been investigated in relation to four different pasture conditions—long grass, short grass produced by gangmowing, grazing by sheep and grazing by resistant cattle.

2. The methods of study of value in this type of field investigation are discussed. It is suggested that the results can be usefully expressed as a series of coefficients

Fig. 1

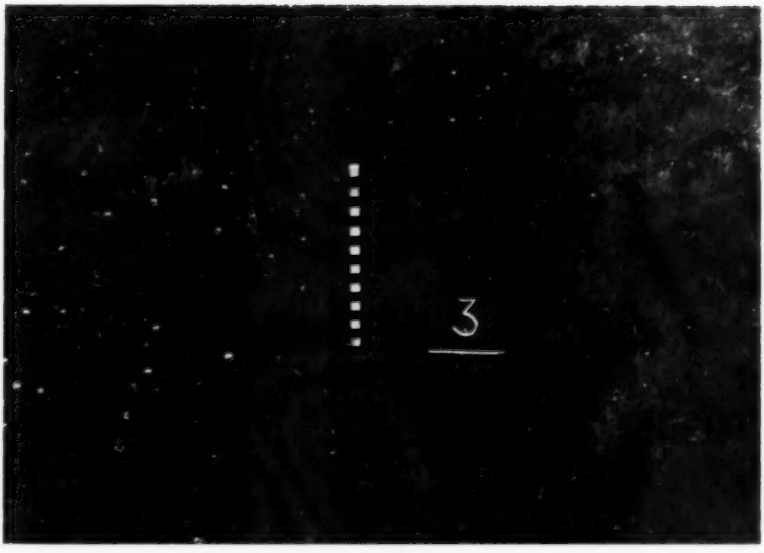

Fig. 2

(*Facing p.* 158)

Fig. 3

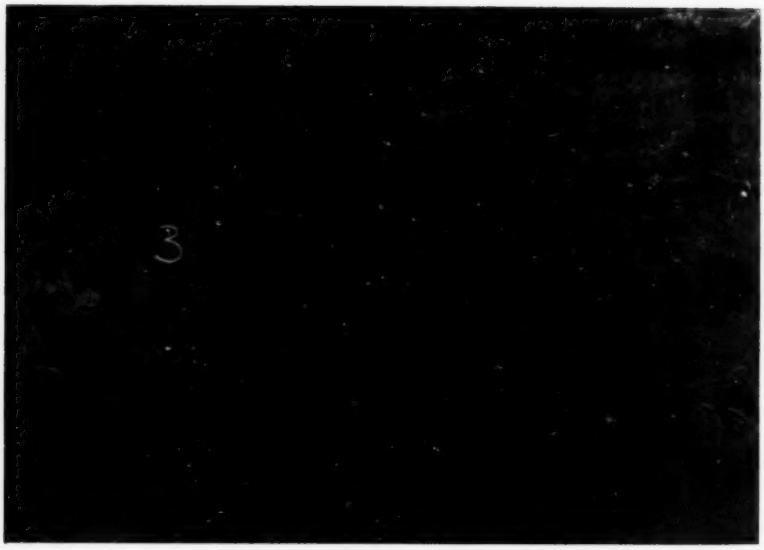

Fig. 4

which summarize the factors involved in the process of transmission. The methods of calculation of these coefficients are described.

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EXPLANATION OF PLATES XII AND XIII

Plate XII

Fig. 1. 'Long' grass (treatment 4) used in Expts. 1 and 2.
Fig. 2. Gangmown plot (treatment 3) used in Expts. 1 and 2.

Plate XIII

Fig. 3. 'Long' grass used in Expt. 3.
Fig. 4. Gangmown plot used in Expt. 3.

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KNEMIDOKOPTES PILAE LAVOIPIERRE & GRIFFITHS, 1951, FOUND ON *PALAEORNIS TORQUATUS* AND *ARA* SP.; WITH A SPECIAL NOTE ON THE BASES OF THE ADSCUTAL SETAE

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(With 7 Figures in the Text)

INTRODUCTION

Lavoipierre & Griffiths (1951) described the female, and Lavoipierre (1953) described the male, of *Knemidokoptes pilae* found on a budgerigar. Later, Oldham & Beresford (1954) and Evans & Browning (1955), respectively, added *Psittacula nipalensis* and *Upupa epops* to the host-list of this species. The present author (1956) has also mentioned the occurrence of *Knemidokoptes pilae* on a budgerigar and a parrakeet, *Palaeornis torquatus*, and an unknown species of the genus *Ara* may now be added to the list.

OBSERVATIONS

Specimens of *Knemidokoptes pilae* taken from *Palaeornis torquatus* did not differ in any significant way from the description of this species given by Lavoipierre & Griffiths (1951) and Lavoipierre (1953), but they differed from *Knemidokoptes laevis*, *K. columbae*, *K. prolificus*, *K. philomelae* and *K. glaberrimus* in having a scaly dorsal pattern and from *K. mutans* in the form of the adscutal setae. Further, *K. pilae* differs from *K. fossor* (Ehlers, 1873) and *K. jamaicensis* Turk, 1950, in having two claws as opposed to four claws on legs III and IV.

A. Knemidokoptes pilae *taken from* Palaeornis torquatus

The measurements obtained from seventy-nine females and sixteen males are given in Table 1. The data given by Henry & Leblois (1924) in their description of head mange of the parrakeets, *Palaeornis alexandri* and *Conorus murinus*, and by Lavoipierre & Griffiths (1951) are added to this table. The measurements given by Henry & Leblois (1924) are included because their description of the clinical appearance of the head mange and the distribution of the mites on the parrakeets suggest that they were dealing with *Knemidokoptes pilae*. Mégnin (1880), describing scaly leg in fowls was the first to mention scaly leg in the budgerigar, which he ascribed to '*Sarcoptes mutans*'.

The dorsal shield in the specimens under consideration in this paper is exactly as Lavoipierre & Griffiths (1951) described it. The epimera of legs I are angulate and the claws on tarsi III and IV are of equal length. The setae on the dorsal surface are situated as they are in *Knemidokoptes mutans*, except that the pair of setae

which is, in *K. mutans*, medial to the large distal setae near to the posterior border of the body beside the anus (Fig. 1*a*), is, in *K. pilae*, posterior to these large setae (Fig. 1*b*). The setae on the ventral surface do not differ from those of *K. mutans*.

MALE. The dorsal surface is smooth, except that the integument at the postero-lateral corners is wrinkled, and there is wrinkling also in the area of the fold at the lateral corners of the proterosoma. The ventral surface is smooth, except for the two areas mentioned by Lavoipierre (1953). The genital apparatus and the setae correspond with Lavoipierre's description and no other setae could be found. The males, however, differ from the drawing given by Lavoipierre (1953, fig. 2), probably because legs I, II and IV in this figure are not exactly figured and the epimera of legs II omitted. All the epimera have a kind of articulation with the legs.

Table 1

Host ...	*Melopsittacus undulatus*	*Palaeornis torquatus*	*Ara* sp	*Palaeornis alexandri*
Data given by	Lavoipierre & Griffiths	Jansen	Jansen	Henry & Loblois
♀♀ length (μ)	286–330*	296–353†	353–403‡	—*
Average length (μ)	—	315	365	315
♀♀ width (μ)	242–286*	252–319†	286–319‡	—*
Average width (μ)	—	272	299	255
♂♂ length (μ)	170–183§	170–198‖	206–214¶	156–163*
Average length (μ)	176	183	210	—
♂♂ width (μ)	98–120§	109–133‖	129–141¶	—*
Average width (μ)	109	118	137	120

* Number of specimens measured: not mentioned.
† Number of specimens measured: 79.
‡ Number of specimens measured: 19.
§ Number of specimens measured: 5.
‖ Number of specimens measured: 16.
¶ Number of specimens measured: 3.

Lavoipierre & Griffiths (1951), describing the female of *K. pilae*, consider that the oval shape of the common base of the two adscutal setae constitutes a striking difference between *K. pilae* and *K. mutans*; and Lavoipierre (1953), describing the male of *K. pilae*, states that the oval base of the adscutal setae is a characteristic feature of the mite. But, as the author (1956) has pointed out, the shape of this base is variable in both the males and females of *K. pilae* and cannot therefore be a feature of diagnostic value.

B. Knemidokoptes pilae *from* Ara *sp.*

Measurements of these, obtained by measuring nineteen females and three males, are given in Table 1.

Female specimens obtained from *Ara* sp. did not differ from *K. pilae*, except in their larger size. In three females the posterior setae on the dorsal surface were arranged as they are in *K. mutans* (Fig. 1), but in one hundred others they were arranged as they are in *K. pilae*. The base of the adscutal setae is variable in shape,

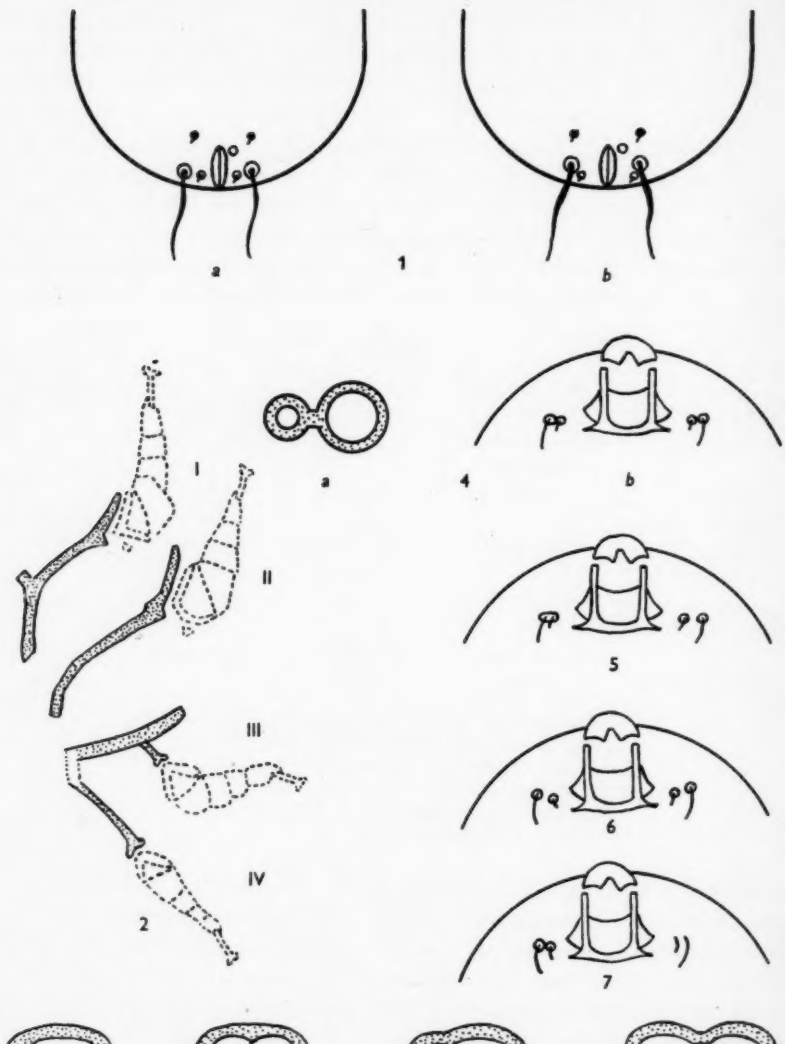

Fig. 1. (*a*) Dorsal view of the anal region of *Knemidokoptes mutans*.
(*b*) Dorsal view of the anal region of *K. pilae*.

Fig. 2. Epimera and legs of a male *K. pilae*.

Fig. 3. Bases of the adscutal setae (see text).

Fig. 4. (*a*) Double base for the adscutal setae. (*b*) Dorsal view of the proterosoma of a female *K. pilae*, showing the dorsal shield and the adscutal setae and their bases.

Figs. 5–7. Dorsal views of the proterosoma of *K. pilae*, showing the dorsal shield and the adscutal setae and the variation in the shapes of their bases.

but the degree of variation in the form of this base in *K. pilae* taken from *Ara* sp. and *Palaeornis torquatus* differs.

MALE. The males agree with the original description except in their larger size and the form of the epimera as mentioned above.

Note on the shape of the base for the adscutal setae

Of 278 mature females of *K. pilae* taken from *Palaeornis torquatus*, 237 had two oval bases. In most of them the ovals were regular (Fig. 3*a*), as Lavoipierre & Griffiths (1951) described, but others showed an irregular inner margin (Fig. 3*b*), or even an oval that was totally irregular (Fig. 3*c*); and one female had one base which was almost double (Fig. 3*d*). In thirty-eight mature females one of the two bases was double (Fig. 4*a*), and in three females both bases were double (Fig. 4*b*). Of fifty immature females, forty-nine had a common oval base on each side and one had two round, separate bases on one side only (Fig. 5). In one of fourteen males one base was double (Fig. 4*a*).

Table 2

Host ...	*Palaeornis torquatus*		*Ara* sp.	
	Mature ♀♀ (%)	Immature ♀♀ (%)	Mature ♀♀* (%)	Immature ♀♀ (%)
Bases	—	—	—	—
2 oval	85·2	98	8·4	26
1 oval, 1 double	13·7	—	15·8	13
2 double	1·1	—	68·5	48
1 oval, 1 separate	—	2	—	—
1 double, 1 separate	—	—	5·3	13
2 separate	—	—	1	—

* In one mature female the adscutal setae had no base.

Of ninety-five mature females of *K. pilae* from *Ara* sp., eight had two oval bases. Among these sixteen bases one was almost double (Fig. 3*d*). In fifteen mature females one base was double, and in sixty-five both bases were double (Figs. 4*a*, *b*). There were also five mature females with, on one side, two round, separate bases (Fig. 5) and one (Fig. 6) in which the bases were separate on both sides, as they are in *K. mutans*. Of twenty-three immature females, the corresponding data were respectively 6, 3, 11, 3, 0. Both oval and double bases were seen in three males (Figs. 3*a*, *b*, *c*, 4). One mature female had, on one side, two adscutal setae without a base, a condition that the author considers to be an abnormality.

The data concerning the shapes of the bases of the adscutal setae of the females are summarized in Table 2, which shows that the variation in the shapes of the bases in specimens taken from *Palaeornis torquatus* is even greater than that in specimens taken from *Ara* sp. These data show that the shapes of the bases have no diagnostic value.

It follows that the only difference between the specimens taken from *Palaeornis torquatus* and *Ara* sp. is the larger size of the specimens from the latter. Possibly

differences in the shapes of the bases are among differences that arise on different hosts. It is therefore concluded that the specimens from *Palaeornis torquatus* and *Ara* sp. are *Knemidokoptes pilae* Lavoipierre & Griffiths, 1951.

CONCLUSIONS

1. A critical study has been made of specimens of *Knemidokoptes* collected from *Palaeornis torquatus* and *Ara* sp., and it is concluded that they are conspecific with *Knemidokoptes pilae* Lavoipierre & Griffiths, 1951.

2. The shape of the bases of the adscutal setae is variable and of very little diagnostic value.

The author is much indebted to J. C. Peters and to Dr E. F. Jacobi and G. M. Smits for the gift of the material used.

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NOTE ON A NEW HOST OF *MYXOBOLUS AEGLEFINI*

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(With 1 Figure in the Text)

Some time ago Mr E. Wilson, of the Marine Laboratory, Aberdeen, brought to notice the abnormal appearance of the cranial cartilage in a plaice (*Pleuronectes platessa* L.) which had been tagged (in St Andrews Bay) and subsequently re-captured (24 miles south-south-east of Arbroath). The fish was a 44·5 cm. long female, 6 years old, with mature ovary and in good condition generally. The cartilage, exposed by the opening of the cranium to remove the otoliths, seemed hypertrophied and full of whitish splotches giving it a mottled appearance. These 'splotches' were formed by irregular, vein-like branching canals, in many places along their length swelling into cyst-like, subspherical cavities up to 3 mm. in diameter. They were filled by a mass of soft substance which was found to be an accumulation of Cnidosporidian spores. Small granules of the substance were also found inside the otic capsules. It is possible, however, that these granules were displaced into the capsules during the opening of the cranium.

This infection of the cranium of plaice with Cnidosporidia has not infrequently been observed by Mr Wilson over a period of about 25 years and can be regarded as relatively common. No records have been kept of its geographical distribution, but it has been found on the east as well as the north and west coasts of Scotland.

Only six species of the fish-inhabiting Cnidosporidia have been found to infest the head. They are shown in Table 1. Only the last of them (*Sphaerospora platessae*)

Table 1

No.	Species	Host	Reference
1	*Myxosoma* (*Lentospora*) *cerebralis* Hofer, 1903	*Salmo fontinalis*	Hofer, 1903; Plehn, 1905.
		Trutta iridea	Hofer, 1903; Plehn 1905.
		T. salar	Hofer, 1903; Plehn, 1905.
2	*Myxobolus aeglefini* Auerbach, 1906	*Gadus aeglefinus*	Auerbach, 1906, 1907
		G. callarius	Reichenow, 1933
		G. merlangus	Reichenow, 1933
		G. esmarkii	Johnstone, 1906
		Molva vulgaris	Reichenow, 1933
		Merluccius merluccius	Johnstone, 1902
3	*M. catostomi* Fantham, Porter & Richardson, 1939	*Catostomus commersoni*	Fantham, Porter & Richardson, 1939
4	*M. dentium*	*Esox-masquinongy*	Fantham, 1939
5	*M. hyborhynchi*	*Hyborhynchus notatus*	Fantham, 1939
6	*Sphaerospora platessae* Woodcock, 1904	*Pleuronectes platessa*	Woodcock, 1904

has been recorded in plaice. Its spores, in common with the other Sphaerosporidae, have a spherical or almost spherical shape. The lenticular shape of the examined spores was, however, typical of the genera *Myxobolus* or *Myxosoma*.

The spores (Fig. 1) were examined directly after their removal from the fish which could have been caught 4–7 days earlier. During most of that period the fish had been kept on ice. The absence of any effective preservative makes the appearance of post-mortem changes and distortions a possibility. The spores were preserved in formalin and re-examined about 3 weeks later in Lugol's iodine stain at a magnification of × 1200, but no changes were observed.

Fig. 1

The spores had two polar capsules situated in one undivided chamber and opening separately to the exterior. Filaments could be seen coiled within the capsules but could not be extruded. A distinct glycogen vacuole filled a considerable portion of the sporoplasm. The walls of the spores were smooth, unsculptured, but with a few folds just observable on the posterior section of the suture line.

The presence of the glycogen vacuole excludes from consideration *Myxosoma cerebralis*, which does not possess such a vacuole. The spores—unless they represent a species which has not yet been described—can belong to one of the four species of *Myxobolus* mentioned in Table 4.

Protozoan parasites of fishes tend to be rather restricted in their tissue specificity and their habitats are of taxonomic value. Of the four *Myxobolus* species *M. catostomi* has been found 'under the epithelium of the floor of the mouth and on the tip of the basihyal bone' (Fantham, Porter & Richardson, 1939), *M. hyborhynchi* in 'a small cyst, 0·4 mm in diameter, embedded in the bone of the posterior end of the mandible' (Fantham, Porter & Richardson, 1939), and *M. dentium* 'in and about

the bases of the palatine teeth' (Fantham, Porter & Richardson, 1939). The habitat of *M. aeglefini* alone corresponds to that in which the spores described in the present paper were found.

Johnstone (1902), describing similar infestation in the hake, *Merluccius merluccius*, mentions also 'hypertrophy of the cartilage of the head', a phenomenon not unlike that observed in the plaice examined by me. The organism involved was, according to Johnstone (1906), *Myxobolus esmarkii*, which was described by him (with Woodcock) from *Gadus esmarkii*. Comparison of the dates of publication of Auerbach's (1906) and Johnstone's (1906) papers shows that these workers were probably unaware of each other's work and that they both described the same parasite, for which the name of *Myxobolus aeglefini* has been generally accepted.

Table 2 compares the dimensions of the examined spores with those of *M. aeglefini* as given by Auerbach (1906).

Table 2

Species	Length (μ)	Width (μ)	Thickness (μ)	Length of polar capsule (μ)
Myxobolus aeglefini	10·8–11·7	9·9–10·4	7·2–9·0	4·5–5·0
Examined spores (range)	10·4–12·1	9·4–11·7	5·9–8·1	4·2–6·8
Examined spores (average of ten measurements)	11·2	10·6	7·0	5·9

Although Auerbach's dimensions represent a spore which is somewhat thicker and with relatively shorter polar capsules than the spores examined by me, there is no incompatibility in size. Auerbach does not state how he has arrived at his figures or how many measurements were taken, but he does mention the great variability in the size of the spores of *M. aeglefini* (Auerbach, 1907).

To check further the identity of the spores, a number of heads of haddock, *Gadus aeglefinus*, were examined. One of them was found to be infested with *Myxobolus* answering Auerbach's description, and was identical in appearance with and well within the size range of the spores found in plaice. No other *Myxobolus* spores were found in any of the heads of haddock examined. Thus it would seem that the spores from the plaice belong to *M. aeglefini*, which therefore also occurs in plaice.

This finding confirms once more the general fact that the sporozoan parasites tend to be tissue- rather than host-specific.

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THE CAPITULUM AND FEEDING MECHANISM OF *DERMACENTOR PARUMAPERTUS* NEUMANN 1901

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(With 34 Figures in the Text)

The so-called rabbit tick *Dermacentor parumapertus* Neumann 1901, is a parasite on hares and rabbits in the south-west of the United States. Philip & Hughes (1953) have already shown that there is a remarkable coincidence of the distribution of the 'black-tailed jack rabbit' and of this tick in the sub-arid and arid zones of the south-west. Study of the disease relations of this species also reveals that there is a natural infection of this species with *Bacterium tularense* of tularemia, a Rocky Mountain fever-like rickettsia and the virus of Colarado tick fever. Moreover, it has been demonstrated that *Dermacentor parumapertus* is capable of experimental transmission of strains of virulent spotted fever, of which *D. andersoni* is the customary vector. This species would thus appear to be a potential source of infection, and in view of our inadequate knowledge of its feeding mechanism a study of the functional elements involved in this process seems desirable. A summary of the literature on feeding mechanisms in ticks has been given elsewhere (Arthur, 1951).

Through the courtesy of Dr Dorrald Allred of the Ecological Unit, Utah, U.S.A., I received a large number of specimens of all stages of this tick, which enabled me to study the external and internal anatomy.

The anatomy of the capitulum was determined from dissections, from caustic potash preparations and from sections of the entire capitulum and the pharynx. Dissection of the upper and lower surfaces was carried out by means of fine pointed needles under a binocular microscope. The integument was chipped off laterally by means of a sharp blade-like needle and the central portion removed from the underlying soft tissue. Such preparations were either mounted in well slides or kept in 70 % alcohol so that examination from all angles was possible. Through the continued and diligent efforts of Mr John Storey a modification of existing techniques for cutting arthropod sections was developed. Storey's technique was as follows:

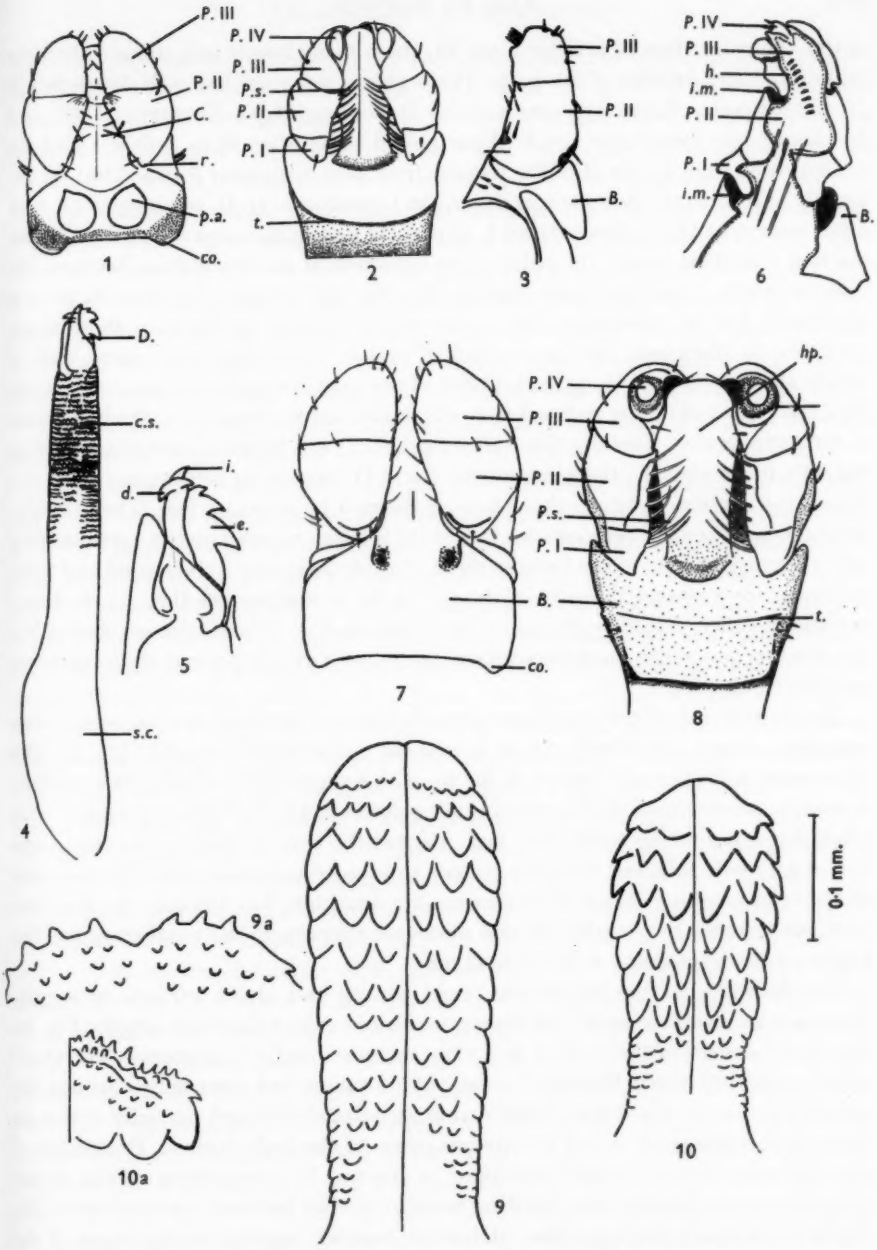
The capitulum was kept in a mixture of absolute alcohol and clove oil for 2–3 hr. and transferred to clove oil for 12 hr. The capitulum was then passed into a supersaturated solution of celloidin in clove oil and kept warm. The solution was thus maintained in a fairly liquid state and was kept therein for at least 2 days. It was then embedded in clove oil celloidin. Subsequently the capitulum was transferred to (*a*) chloroform, and (*b*) to a solution of chloroform and paraffin wax (melting-point, 56° C.) at 39° C. for about 36 hr. During this time the solution was placed in a vacuum pump for 30 min. Embedding was completed in three baths of paraffin wax (melting-point 56° C.) for ½ hr. each. This wax-celloidin ribbons perfectly providing that adequate wax is left surrounding the celloidin block.

Stretching of the sections was completed by the use of Ruyter's fluid (acetone, 2 c.c., H_2O, 8 c.c., methyl benzoate, 1 drop, egg albumen, 2–3 drops). These sections were stained with Mallory's triple stain. Storey's method has also proved effective in cutting sections of chilopods and myriapods.

EXTERNAL ANATOMY

The capitulum of the female consists of a median ventral hypostome flanked on each side by a four-segmented palp arising from a broad basis. A pair of long cylindrical shafts, the chelicerae, overlies the hypostome dorsally and passes back through the body of the basis. Dorsally (Fig. 1) the latter is broader than long, this ratio being as 3·2:1·4; the lateral margins are straight, converging strongly to the base of the cheliceral sheaths antero-laterally. The postero-lateral margins are broadly rounded to form the cornua: the width at the base of the latter is greater than the length and the apex is rounded. The surface is generally flattened except behind the porose areas where it is undulate, and punctations are scarce or absent; the lateral and posterior margins are more deeply pigmented than the rest of the dorsum. The large, pear-shaped porose areas are deeply sunk into the integument, with their axes set obliquely to the long axis of the body. The interporose distance is variable, being less than half the width of the porose areas in most specimens. The dorsal ridge is salient and strong. The basis is broad ventrally (Fig. 2), with a prominent curved postero-ventral ridge, and in front of this the surface is flat nearly to the hypostomal base where it passes into a crescentic depression. The basis behind the ridge slopes down quite strongly to its posterior margin. Both surfaces, when seen in side view (Fig. 3), converge distally, the lower surface sloping more acutely than the upper. Ventro-laterally the basis bears three or four short, sharp, pointed hairs.

The palps are longer and narrower in *D. parumapertus* than in related species. Articles I and IV are visible only from the ventral surface. The median face of article II, when seen from above, is straight and in the non-feeding female is oblique to the long axis of the body. Article III, similarly, has a straight mesial profile which lies roughly parallel to the afore-mentioned axis. The apex of article III is subangular near the mid-line becoming gently convex laterally. Article II is widest apically and then passes back obliquely at about one-third of the distance from the distal end, to form a moderate, almost pointed postero-dorsal ridge with rounded edges. Small faint punctations may be present on the palp, but in some specimens these are absent. A few short hairs are present laterally and apically. Article I is a triangular plate with a salient postero-ventral margin. Ventrally article II is longer than broad, and somewhat swollen near its apex; article III is, however, broader than long. Meso-ventrally article III forms a triangular, flattened area which extends back beyond half the length of this article. The apical margin of article III is rounded at its junction with article IV; the articulating membrane between these articles is extensive. The apex of article IV bears half a dozen sensillae, which have rounded tips, and three to four moderately long pointed hairs around the periphery. A series of curved, palpal setae arises from the mesial border



Figs. 1–10*a*. *Dermacentor parumapertus*. Fig. 1. Basis capituli of female, dorsal view. Fig. 2. Basis capituli of female, ventral view. Fig. 3. Basis capituli of female, lateral view. Fig. 4. Cheliceral shaft of female. Fig. 5. Digit of female. Fig. 6. Longitudinal section through the palp along the median line. Fig. 7. Basis capituli of male, dorsal. Fig. 8. Basis capituli of male, ventral view. Fig. 9. Hypostome of female. Fig. 9*a*. Tip of hypostome enlarged. Fig. 10. Hypostome of male. Fig. 10*a*. Half tip of hypostome enlarged.

of the ventral surface of articles I and II, these being longer and thicker than the other hairs and bristles of the palp. These palpal setae are less well developed in *D. parumapertus* than in species such as *D. circumguttatus* Neumann, 1897, and *D. rhinocerotis* Gerstäcker, 1873. Four palpal setae arise from article I and six similar setae from article II of the female; they pass diagonally forward but do not usually extend to the mid-ventral line of the hypostome. In *D. parumapertus*, as in other species of this genus, articles I, II and III do not move on each other. When the tick is sucking blood, the palps move outwards at an articulation between the base of article I and the basis capituli (Fig. 6), and at this connexion there is a prominent fold of intersegmental membrane. The walls of the palp show much variation in thickness; the upper wall of article I and the lower basal wall of article II are stout, and along the mid-line of the palp the walls become so thickened that the palpal cavity is reduced to a relatively narrow connexion which becomes wider peripherally. Distally the walls of article II are thinner, becoming more so laterally in article III. Between articles I and II there is no intersegmental membrane, the division between them being indicated by a suture line. The intersegmental membrane between articles II and III is much reduced on the upper surface but is distinguishable on the lower surface. The internal faces of the second and third segments are concave; the palp is broader on its ventral surface than on its dorsal surface and the overlap is greater on the upper surface. When apposed during the non-feeding period the mesial margins of the palps partially protect the hypostome and the chelicerae.

The ventral wall of the capitulum extends forwards between the palps to form a spatulate-shaped hypostome, i.e. it is broader apically than basally (Fig. 9). The hypostome is in general rounded at the tip with a slight indentation in the midline. A corona, consisting of three rows of small pointed teeth, lies behind the apex, with small distal projecting teeth (Fig. 9*a*). Behind the corona there is one transverse row of 4/4 teeth, followed by about sixteen to seventeen lateral teeth. The majority of the corresponding mesial rows present 3/3 dentition, but towards the base the teeth are reduced to crenulations and moreover thin out to the basal margins. The length of the hypostome is about 0·43 mm.

The chelicerae of the female are broad, strong and about 1·0 mm. in length, becoming swollen proximally for about one-third of the total shaft length (Fig. 4). The distal surface of the shaft is reticulate but proximally it is smooth. The chelicerae emerge anteriorly through the capitular foramen and posteriorly through the occipital foramen. Each is enclosed within an outer sheath and partially within an inner sheath (see later) which are invaginations of the body cuticle. Protrusion of the chelicerae under normal conditions is effected by contraction of the dorso-ventral somatic muscles, the resulting pressure within the body cavity forcing the shafts out through their sheaths. Retractor muscles, inserted on the bases of the chelicerae, pass postero-dorsally and are attached to the lower surface of the posterior margin of the scutum. Movement in a horizontal plane is prevented by the close apposition of their basal positions and of internal guiding channels formed by the subcheliceral sheaths, to be described later.

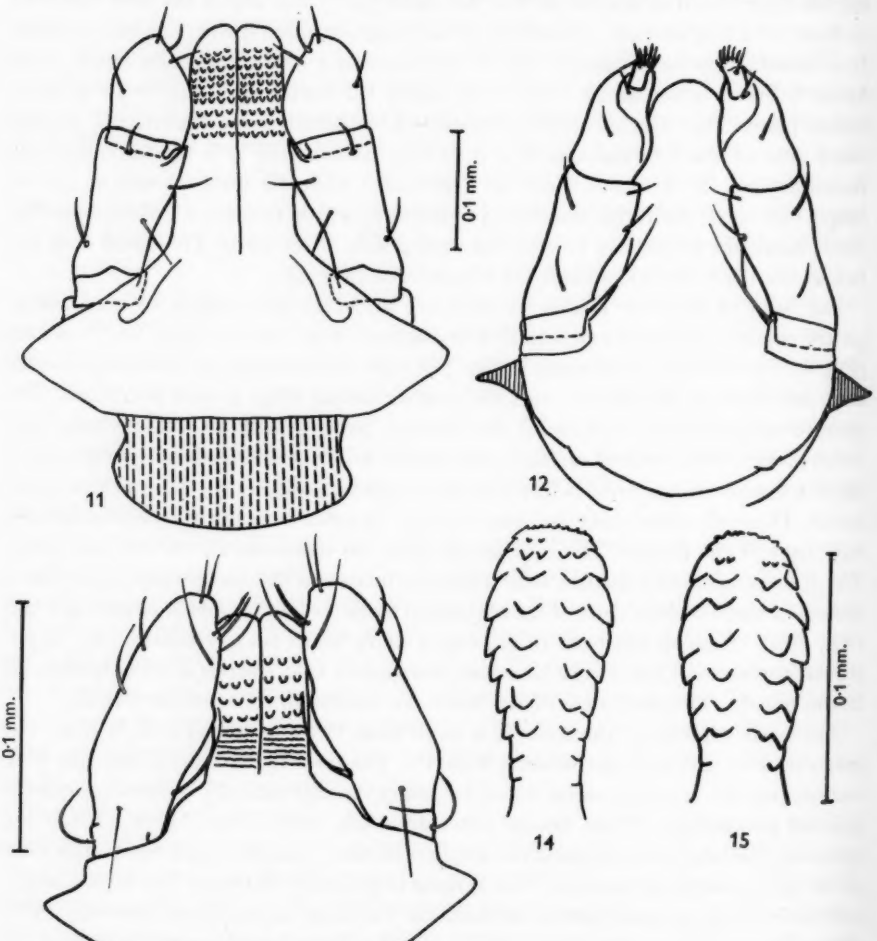
The cheliceral shaft bears a denticulate digit at its apex which moves by the

action of antagonistic muscles arising within the shaft. The cavity of the expanded proximal portion of the shaft is completely filled by these flexor and extensor muscles. Tendons of these muscles pass forwards through the distal portion of the shaft to their points of insertion on the apical digit. The digit conforms to the chelate type found in arachnids, but the usual pincer-like organ has been modified to form a cutting surface. It consists of a triangular plate drawn out into a point. It is attached by its expanded base to the extremity of the cheliceral shaft which forms a broad articulatory surface on which the entire digit moves in a meso-lateral plane. The external article is broad and consists of three sharp teeth. On the outer side of the internal article and flexibly attached to it is the movable bifid dorsal article. It does not, however, articulate with the internal article. In all stages the outer cheliceral sheath is produced forwards to form a reticulate sculptured hood-like protective lobe on the mesial side of the digit. This hood does not enfold the digit when the chelicera is extruded (Fig. 5).

The basis of the male is also rectangular: the sides are straight, or more rarely gently curved and pass back into rounded cornua which are about as broad as long (Fig. 7). The surface of the basis is flat. The palps of the male are relatively shorter than are those of the female, and the postero-dorsal ridge is well developed. The postero-ventral ridge is elevated and salient, passing back to a declivitous posterior margin. The ventral surface, divided into anterior and posterior portions by a shallow transverse groove, is flat and slopes gently towards the base of the hypostome. There are a few scattered punctations. In other respects it agrees essentially with that of the female. The hypostome (Fig. 10) measures about 0·28 mm. long. The apex consists of a median lobe which undulates to the margin and comprises a corona of three to four rows of short pointed teeth, which project forwards in front (Fig. 10*a*). Nine to ten rows of 3/3 equal sharp teeth occupy most of the hypostomal surface and nearer the base pass marginally into a series of crenulations, as in the female. The chelicerae of the male are similar to those of the female.

The basis capituli of the nymph is more than twice as broad as it is long; the mean ratio of fifty specimens being 3·25:1·5. The posterior margin is straight with convex postero-lateral margins which become extended laterally to form prominent pointed projections. These lateral extensions are visible from below. From the apices of the lateral extremities the straight anterior margins converge to the sides of the outer cheliceral sheaths. The surface is generally flattened but with a slight curvature to the pointed lateral extremities. The basis is also broad ventrally, with a broadly rounded postero-ventral ridge which is more heavily sclerotized than the rest of the basis. Postero-laterally the ridge is drawn out into small projections, and towards its lateral extremity a single hair of moderate length arises on each side (Fig. 12). Article I is only partially visible from above, being overlapped by the antero-lateral extensions of the basis. Article II attains its greatest width just behind its distal end and narrows basally. The lateral profile undulates and near its base there is a single curved hair. Mesially the profile is asymmetrically convex, with one or two long hairs directed to the mid-line. Article III is considerably shorter than article II and, as seen from above, is cone-shaped, bearing one hair laterally and three or four similar hairs near the tip. The lengths of articles II and III are

0·14 and 0·09 mm., respectively. The outer cheliceral sheath is smooth for about half its distance forward from the tectum, but in front it is covered with transverse rows of small backwardly pointed elevations. The cheliceral shaft closely resembles that of succeeding stages and measures 0·41 mm. long. The hypostome is spatulate



Figs. 11–15. *Dermacentor parumapertus*. Fig. 11. Basis capituli of nymph, dorsal view. Fig. 12. Basis capituli of nymph, ventral view. Fig. 13. Basis capituli of larva, dorsal view. Fig. 14. Hypostome of nymph. Fig. 15. Hypostome of larva.

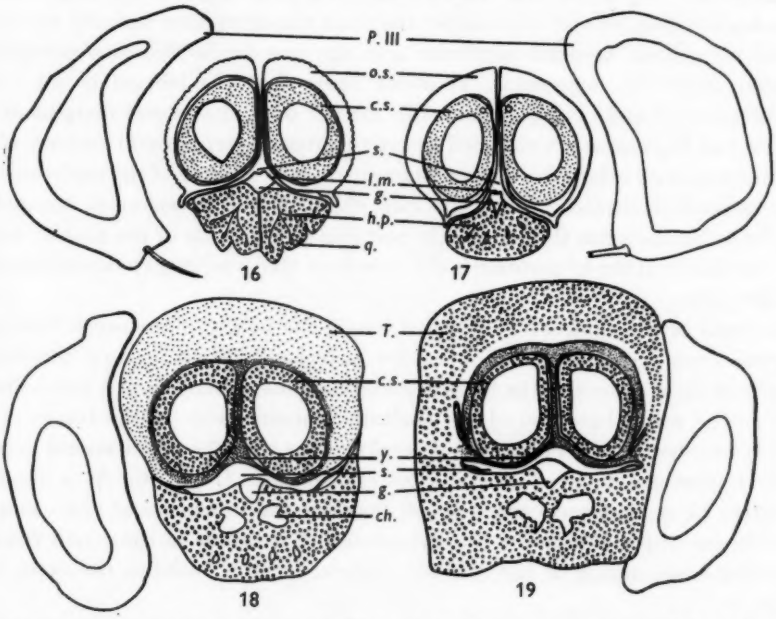
(Fig. 14), a corona is present and the dentition from apex to base is one row of 3/3 teeth, five to six rows of 2/2 teeth, and basally there are two lateral crenulations. The length of the hypostome is 0·11 mm.

The larval basis is similar to that of the nymph in all essential features (Fig. 13). Such differences as do occur relate to the palp. The hairs on the latter are long and

thin, being relatively longer than in the succeeding stages. No suture line is evident between articles II and III; the combined length of articles II and III is about 0·11 mm. The hypostome is spatulate; the dentition consists of six to seven rows of 2/2 teeth surmounted by an apical corona.

INTERNAL ANATOMY

The capitulum is divisible into (i) an extra-basis or pre-oral region comprising the hypostome, the distal ends of the chelicerae and the palps, and (ii) the basis capituli, where partial or complete morphological union of these parts has taken place. When the tick is not feeding the palps are closely apposed and partially



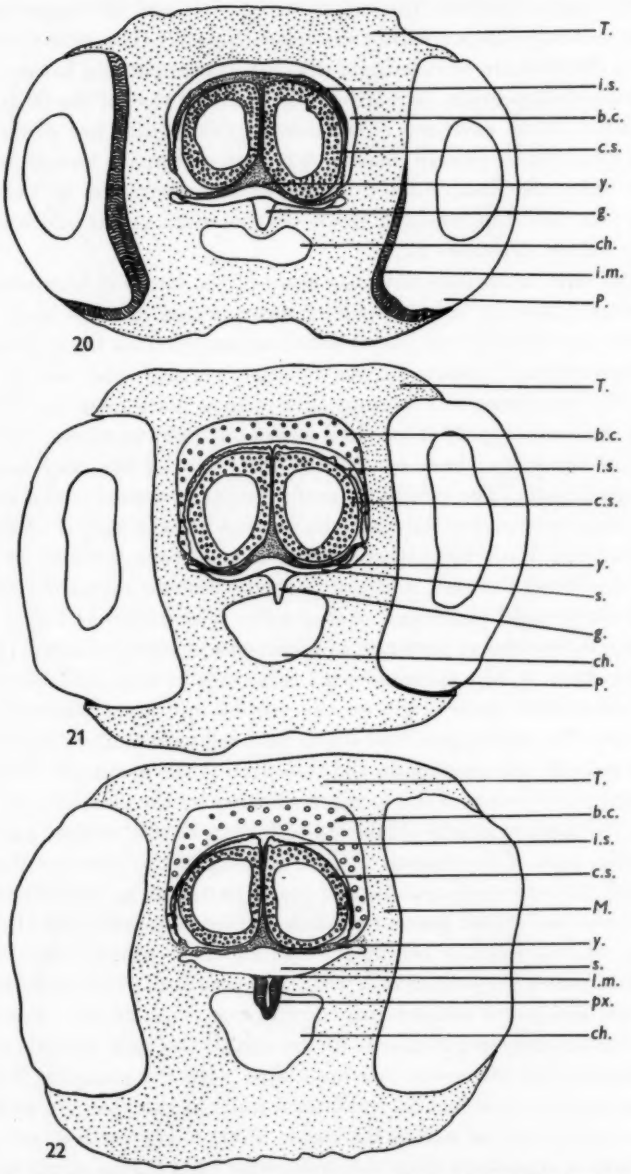
Figs. 16–19. *Dermacentor parumapertus*. Figs. 16–18. Transverse sections of the extra-basis region showing only one palp in each case. Fig. 19. Transverse section through the region of fusion of the tectum with the hypostome.

overlap the chelicerae and the hypostome thus protecting a salivary channel and a food groove on the hypostome. The hypostome, when seen in section, is broader than long at the apex and is divided into two halves by a vertical suture line. The upper surface is indented along the mid-dorsal line to form a V-shaped depression, the hypostomal groove (Fig. 16). Lateral to this groove the upper surface of the hypostome slopes downwards and outwards to its greatest width, then below it slopes inwards to the mid-ventral line and bears three denticles on each side. An elevated membrane, or labrum, which overlies the hypostomal groove, is continued laterally and appears to be closely adherent to the surface of the hypostome. The chelicerae lie above the hypostome. On their mesial surface the sides of the shafts are straight, being broadly rounded ventrally and mesially; the

dorso-mesial profile is more angular. At this level I have failed to find an inner cheliceral sheath; the dorsal and lateral margins of the outer cheliceral sheath are thick and thrown into pronounced ridges; ventro-laterally it is drawn out into a small lobe which overlaps the side of the hypostome. Between the shafts and ventrally this sheath is thin and non-ridged. Sections through this extra-basis region show that there is a close fit between the lower surfaces of the chelicerae and those of the upper surface of the hypostome (Figs. 16–18). The salivary channel is a triangular lumen (Figs. 16, 17) which lies above the labrum and between the lower and inner rounded margins of the cheliceral sheath. As a consequence of this the salivary channel opens at the tip of the hypostome above the labrum when the chelicerae are fully protruded. This pattern is typical of the whole length of the extra-basis region, except that nearer the basis the depression beneath the hypostomal membrane becomes narrower and the membrane becomes secondarily elevated above the depression. It seems likely that the labrum is not a soft structure but is quite rigid. About 10μ farther back the lateral margins of the hypostomal depression are elevated above the general hypostomal surface, which is concave on each side of the former. The ventral convex faces of the chelicerae and their associated sheaths fit snugly into these lateral hypostomal concavities (Fig. 18). Channels run through the hypostome on each side of the median suture line. At this level the hypostome is still free from the overlying cheliceral complex and the palps.

The basis of the capitulum is formed by (1) the fusion of the much thickened outer cheliceral sheaths to form the dorsum (or tectum), (2) the fusion of the median margins of these same sheaths to form a vertical plate arising from a subcheliceral plate (=the ventral portion of the cheliceral sheath), and (3) the fusion of the lower lateral borders of the outer cheliceral sheaths with the upper lateral margins of the hypostome to produce the sides of the basis (Fig. 19). As a result of the latter (3) a transverse space is left between the subcheliceral plate and the more mesial upper surface of the hypostome. This space is the much widened backward continuation of the salivary channel already noted in the extra basis region.

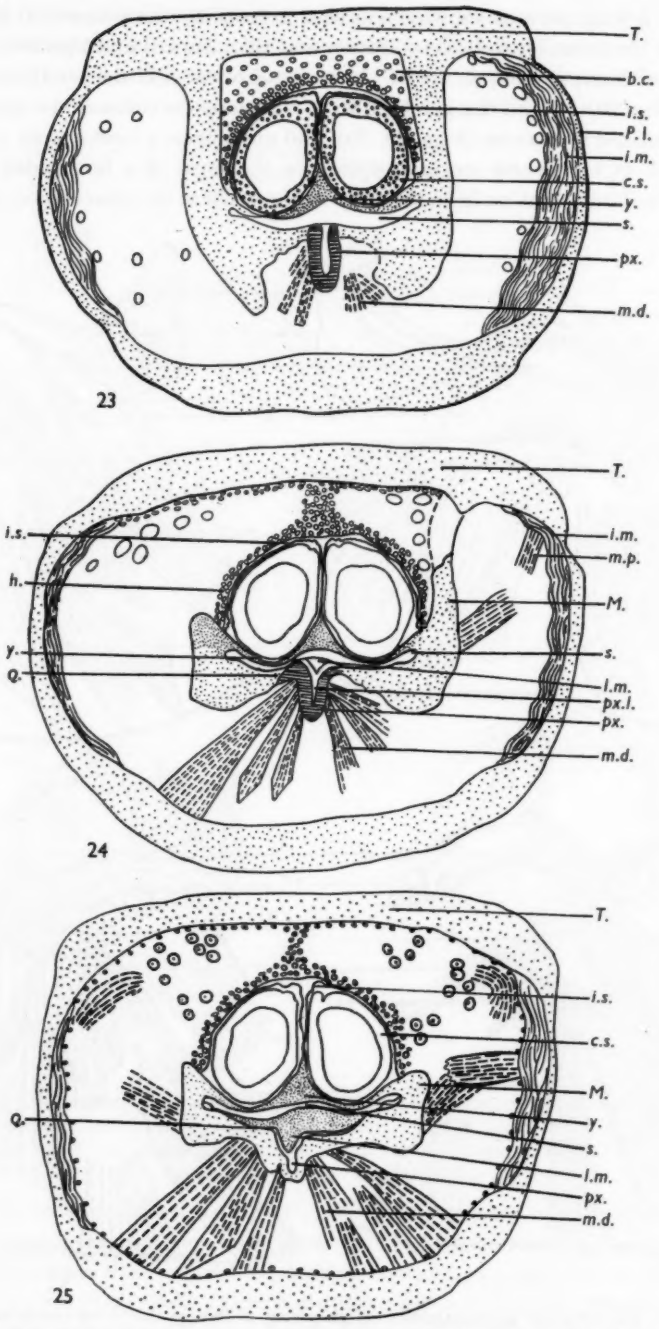
The tectum becomes progressively thicker proximally and three 'layers' are recognized by staining with Mallory's stain. The outermost and thickest layer stains red and is of a granular consistency; an intermediate layer which does not stain as deeply; and an inner, moderately thick, regular, red-staining layer which becomes the inner cheliceral sheath. The latter is soon separated from the rest of the tectum for most of its length by a space, narrow at first, but becoming broader posteriorly: this is a forward continuation of the body cavity. Ventro-laterally the inner cheliceral sheath arises from the lateral walls of the tectum (Fig. 19 *et seq.*) as a thin, much folded membrane and stains blue with Mallory's stain. At the distal end of the basis this membrane is short, continuing into a more heavily sclerotized dorsal and dorso-lateral portion. Posteriorly the inner sheath gradually becomes entirely membranous and much folded (Figs. 21–25). Between the tectum and the inner cheliceral sheath there is a cavity which is lined with epithelium. This epithelial layer becomes limited to the sides and above the inner cheliceral sheaths



Figs. 20–22. *Dermacentor parumapertus*. Transverse sections through the basis to just beyond the incorporation of the palps with the basis. For explanation see text.

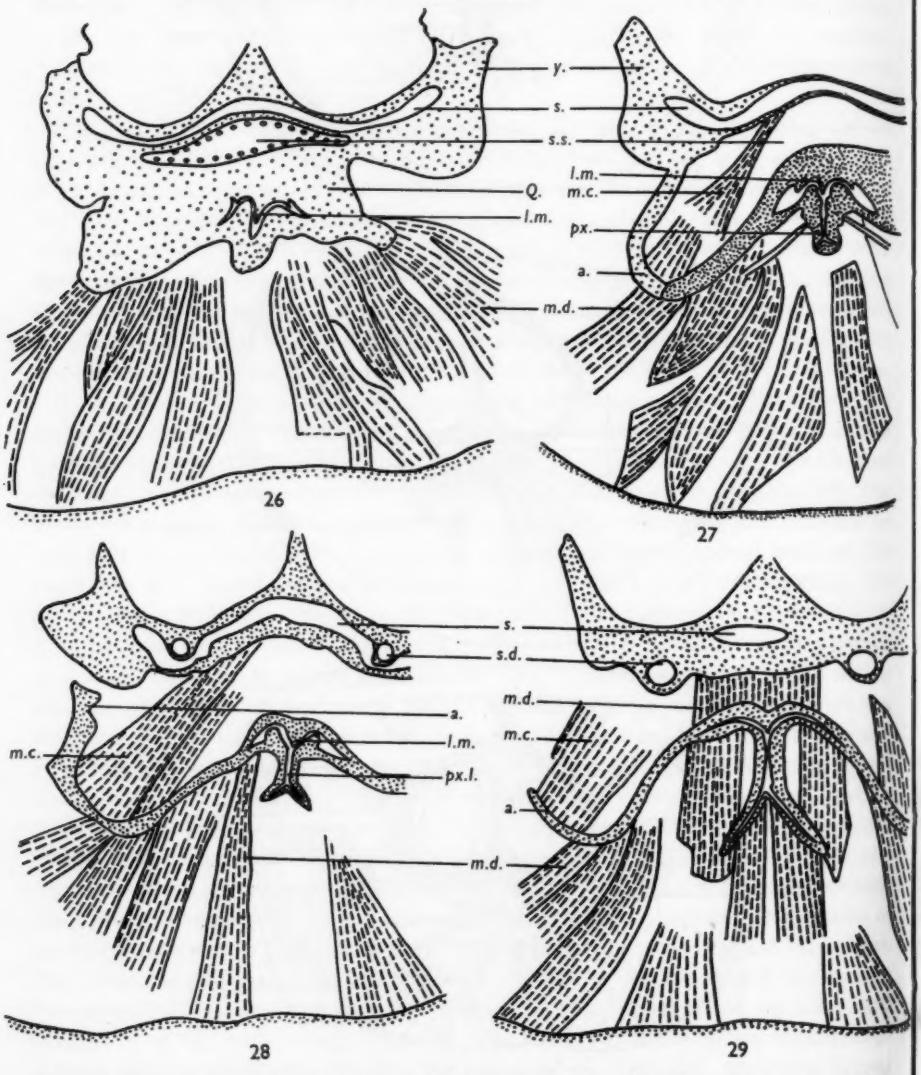
and eventually it is continued upwards as a median layer which ultimately joins with the epithelium underlying the tectum (Figs. 24, 25). The transverse space of the salivary channel between the subcheliceral plate and the upper surface of the hypostome, becomes deeper within the basis (Fig. 22). The lateral extremities of the salivary channel are rounded and divided into upper and lower cavities by a membrane which appears to be a lateral extension of that of the labrum (Fig. 21). The function of these cavities at the rounded extremities has so far eluded me. Below the hypostomal groove there is a rectangular space formed by the fusion of the two channels already noted in the extra-basis region of the hypostome. Posteriorly this cavity increases in size in all directions and is a forward extension of the body cavity (Figs. 20–25).

The palpal base articulates with the body of the basis by blue-staining cuticle (Fig. 20) before the palp becomes solidly incorporated into the body of the basis (Figs. 21, 22). At this level the palpal base is separated from the cheliceral complex and the hypostomal groove by an upright vertical wall about 6–10μ long (Figs. 21, 22). In subsequent sections this vertical plate loses its connexion with the floor of the basis (Fig. 23 *et seq.*) and passes obliquely upwards, thereby making the cavity of the palpal base continuous with that of the very much enlarged subpharyngeal cavity. The subpharyngeal cavity is so named at this level (Fig. 23) because of the presence of dilator muscles attached to the wall of what is functionally the pharynx. With the disappearance of the lower portions of the vertical plates the cheliceral complex and the pharynx become supported by the dorsal portions of the vertical plates and of transverse sclerotizations which at this level consist of the subcheliceral plate and of transverse pharyngeal struts (Fig. 23). The V-shaped pharynx is here supplied with three sets of oblique dilator muscles on each side which have their origin on the ventral and ventro-lateral walls of the basis (Fig. 24). The pharyngeal wall stains dark red with Mallory's triple stain. No constrictor muscles are present on the fore-part of the V-shaped pharynx, and it would appear that closure of the lumen is effected by the elasticity of the pharyngeal walls. The labrum is here thickened to form a broad 'wedge' partially fitting into the upper part of the lumen. At the opening of the pharynx the wedge is a thickening of the membrane, but farther back the membrane thickens into a transverse sclerotized bar which passes outwards to unite laterally with the pharyngeal struts (Figs. 24, 25). Slightly behind the opening of the pharynx the dorsal portion of the vertical plates loses connexion with the upper wall of the basis and its upper margin passes backwards and obliquely downwards (Figs. 24, 25). Accordingly, the only sclerotized supporting elements in the rest of the basis are the subcheliceral plate and underlying transverse bar associated with the pharynx. Three pairs of powerful muscles are inserted on the lateral walls of the pharynx and these have their origin on the ventral wall of the basis. Some 8–10μ farther back the floor of the salivarium is separated from the transverse pharyngeal struts by a forward outgrowth of the body cavity (Fig. 26). The 'wedge' then becomes tripartite so that its median projection fits into the lumen of the pharynx, whilst its lateral projections lie close against the upraised margins of the cleft. The cavity beneath the salivarium floor becomes wider and deeper more posteriorly, and is accordingly



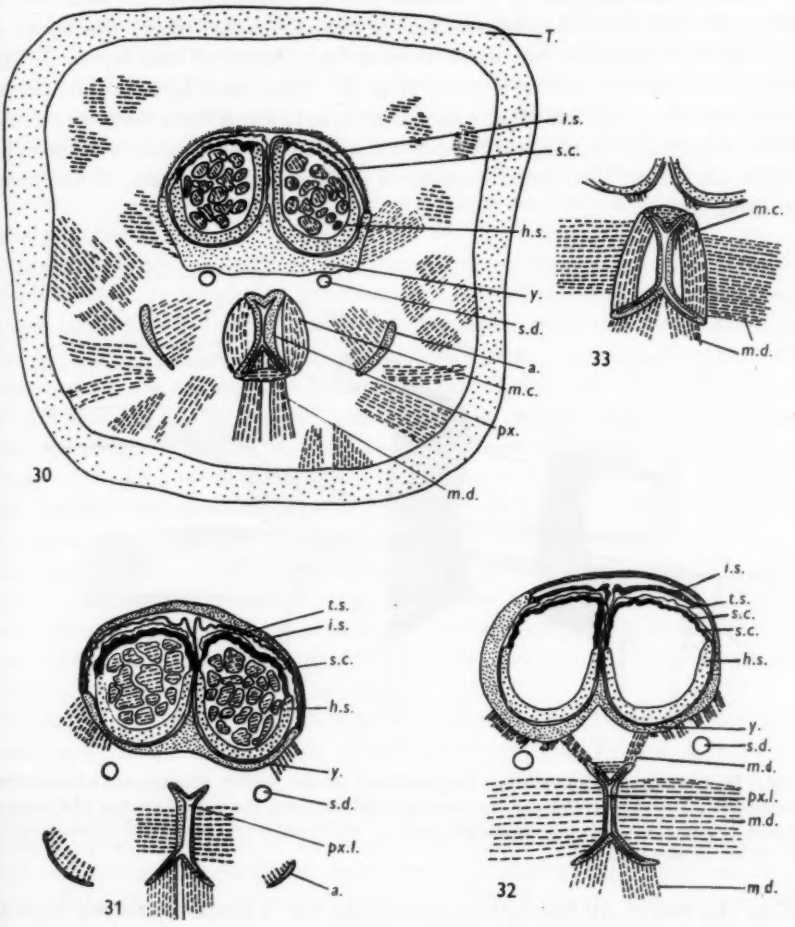
Figs. 23–25. *Dermacentor parumapertus.* Transverse sections through the basis beyond the incorporation of the palps with the basis. For explanation see text.

flanked by a wing plate or ala which, as seen in section, passes outward and downward from the subsalivarial sclerotization and then inward and upwards (Fig. 27) to merge with supporting struts of the now narrower and deeper three-pronged wedge. Some 20 μ behind this level the wing plate loses its connexion with the overlying sclerotized structures (Figs. 28, 29), and now forms a backwardly projecting wing which in transverse section appears in the form of a horizontal S-shaped plate. A similar separation from the supporting struts of the pharynx occurs a little



Figs. 26–29. *Dermacentor parumapertus.* High-power drawings based on transverse sections through the region immediately below the sub-cheliceral plate to show the origin of the wing plates and their associated musculature. For explanation see text.

farther back, so that in section the sclerotization appears to lie free within the posterior part of the basis (Fig. 30). Muscles arise from the inner face of the elbow of the flanking wall and cross to the floor of the salivarium. Antagonistic muscles are inserted on the outer wall of the elbow and pass obliquely backwards to the



Figs. 30–33. *Dermacentor parumapertus.* Fig. 30. Transverse section through the basis showing constrictor muscles around the pharynx, the posterior extension of the wing plates, and the membranous dorsal and dorso-lateral portions of the chelicerae. Figs. 31–33. Transverse sections through the chelicerae and pharynx at their posterior limits.

ventro and ventro-lateral walls of the basis. These muscles I refer to as the elevator and depressor muscles respectively of the wing plate or ala. Five or six bands of dilator muscles are inserted on the pharynx and pharyngeal struts at this level, in addition to two pharyngeal ligaments which pass out laterally. Similar elevator and depressor muscles arise from the flanking wall farther back even when connexion with the subcheliceral sclerotizations is no longer evident; in other words,

the salivarium-alar muscles pass obliquely forwards. The posterior end of the salivarium is now evident as a narrow elongate oval space embedded within the thick subcheliceral plate. The salivary ducts entering into the salivarium are also incorporated into the subcheliceral plate for a short distance. The pharynx becomes bifid ventrally and supplied with vertical dilator muscles (Fig. 29) and this level corresponds with the proximal swelling of the chelicerae, where the upper and lateral parts of the shaft are much thinner and consist of two layers of 'blue' staining cuticle (with Mallory's stain) (Fig. 32). The outer layer is thin (tertiary sheath) and very much folded, and the inner layer is about three times as thick and lacks the pronounced foldings of the outer layer. Entire chelicerae, when seen from the side, show that this region consists of a short semilunar area of less heavily sclerotized tissue.

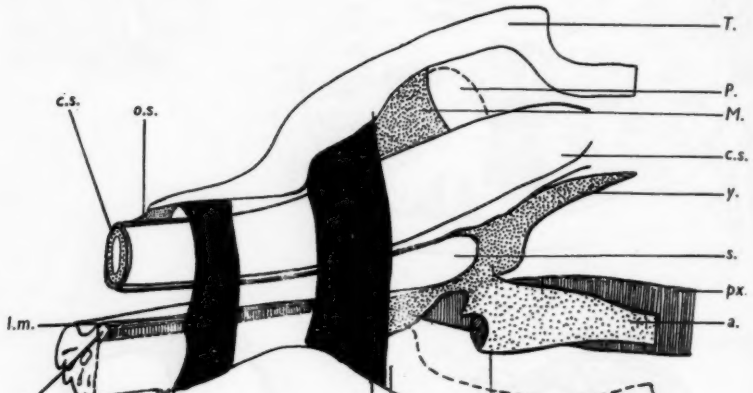


Fig. 34. *Dermacentor parumapertus.* Sagittal half of the female capitulum, reconstructed. The solid black portions represent the vertical wall between the mesial part of the basis and the palps before they fuse. The numbers refer to sectional levels, for which illustrations are given earlier in the text.

When the wedge (of the labrum) overlying the V-shaped pharynx separates from the wall of the wing plate it becomes the dorsal wall of the pharynx (cf. Figs. 29, 30). The wedge is still apparent and subdivides the pharyngeal lumen into two arms. Dilator muscles originate on the subcheliceral plate and are inserted on to the apices of these dorsal pharyngeal arms. Similar muscles supply the ventral arms, and broad dilator muscle bands are inserted on the sides of the pharynx passing horizontally to the wall of the basis.

The pharynx is a powerful suctorial organ, approximately 0·5 mm. long, and is pisiform in shape. The walls consist of heavily sclerotized plates, connected by flexible membrane, arranged in the form of two Y's placed with their bases together. The sides consist of a pair of large concave plates and the dorsal and ventral sur-

faces of two pairs of smaller plates. In the absence of constrictor muscles at the anterior end of the pharynx, the active movements are those of opening by the dilator muscles and closure by a recoil of the pharyngeal walls which occlude the opening. The 'wedge', in view of its shortness, would at this level seem to exert only a limited valvular action. Farther back at the level of the alae the closing and opening mechanism appears to be reinforced by the subsalivarial-alar muscles (Figs. 27–29). By their contraction they would tend to draw the 'elbow of the ala' upwards and depress the wedge into the pharyngeal lumen. Conversely, the ventral basis-alar muscles would depress the elbow, thus elevating the wedge. All the muscles attached to the alar processes pass obliquely forwards. When the pharynx has completely separated from the alar plates posteriorly the muscles consist of the following: (i) the lateral dilators, which are large and have their insertion on the lateral pharyngeal plates and their origin on the lateral wall of the basis; (ii) ventral dilators which consist of two groups arising from the ventral wall of the basis and which are inserted on to the ventral walls of the pharynx; (iii) the dorsal dilators, two relatively small muscle bands arising from the dorsal pharyngeal arms, have their origin on the subcheliceral plate; (iv) the four constrictor muscles, which are arranged so that they connect the arms of the pharynx and alternate with the dilator bands. These constrictor muscles make their appearance behind the posterior limit of the alae. The oesophagus arises from the pharynx as a narrow tube, which bends sharply downwards as it nears the brain. It enters the brain ventrally and passes through it at an angle of 45° and emerges dorsally from it at about two-thirds the distance towards its posterior end. From here the oesophagus flattens out and enters the stomach as the proventricular fold. Histologically the cells are large, irregular and somewhat angled and rest on a thin basement membrane. The nuclei are of moderate size and irregular in shape. Overlying these cells and bounding the lumen of the cavity is a very thin chitinous layer which is continuous with the walls of the pharynx.

The salivary ducts, for some distance beyond the end of the salivarium, pass in the substance of the subcheliceral plate and on emerging from it diverge in the direction of the scapulae. The ducts open into the salivary glands which in unfed ticks extend from the scapular angles to the stigmal plates. Each gland consists of a mass of spherical alveoli of approximately 0·02–0·04 mm. in diameter. These alveoli are of two different types. In the anterior third there is an indefinite peripheral cellular structure with fibrils radiating in the direction of the lumen and, as far as can be ascertained, they end freely. The nuclei are small and appear to be near the lumen. The second type of alveolus forms the bulk of the gland; the cells are relatively large, with large basal nuclei and they may contain ferment granules or appear as pale reticulate cells devoid of granules.

SUMMARY

The external structure of the capitulum of all stages of *Dermacentor parumapertus* is described in detail. The capitulum is divisible into an extra-basis and basis regions. The former consists of a dorsally grooved hypostome or food channel

overlapped by a labral membrane, along its whole length. The salivary channel lies above the labral membrane, being bordered laterally by the meso-ventral margins of the outer cheliceral sheaths. Within the basis a vertical wall separates the base of the palpal cavity from the rest of the basis. This wall is produced backwards to form a wing or ala on either side of the pharynx and to it are attached dilator and constrictor muscles. Opening of the fore-part of the pharynx, i.e. where it is in the form of a V-shaped depression overlapped by a wedge, is effected by ventral and ventro-lateral dilator muscles, and closure by the elasticity of the pharyngeal walls.

The pharynx, oesophagus and salivary glands are described.

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KEY TO ABBREVIATIONS

a.	wing plate or alar plate	*m.p.*	palpal muscle
B.	basis capituli	*o.s.*	outer cheliceral sheath
b.c.	body cavity	*P.*	palp
C.	chelicerae	*P. I–IV*	palpal articles *I–IV*
c.s.	cheliceral shaft	*P.s.*	palpal setae
ch.	hypostomal channel	*p.a.*	porose areas
co.	cornua	*px.*	pharynx
D.	digit	*px.l.*	pharyngeal lumen
d.	dorsal process	*Q.*	pharyngeal struts
e.	external article	*q.*	denticle
g.	hypostomal groove	*r.*	postero-dorsal extension of article II
h.	epithelium	*s.*	salivarium
hp.	hypostome	*s.c.*	membranous part of cheliceral shaft
h.s.	more heavily sclerotized part of cheliceral shaft	*s.d.*	salivary duct
i.	internal article	*s.s.*	subsalivarial cavity (= forward continuation of body cavity)
i.m.	intersegmental membrane	*T.*	tectum
i.s.	internal cheliceral sheath	*t.*	transverse ridge
l.m.	labrum	*t.s.*	tertiary cheliceral sheath
M.	vertical wall between palp and the mesial portion of the basis	*y.*	subcheliceral plate
m.c.	constrictor muscles		
m.d.	dilator muscles		

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SOME FIELD AND LABORATORY OBSERVATIONS ON THE BRITISH HARVEST MITE, *TROMBICULA AUTUMNALIS* SHAW

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Among groups of medically important arthropods, Trombiculid mites have been among the most refractory to culture in the laboratory. The principal difficulty in all cases so far investigated has been the provision of a suitable food for the free-living postlarval stages.

Within the last decade, however, a number of workers in different parts of the world (Ceylon, India, Japan, Malaya and the United States) have reported the rearing of a number of different species through at least one complete generation. In all the reported instances, the eggs, or other inactive stages of various small arthropods, have been found to provide the most acceptable and practicable source of food for the nymphal and adult stages of the mites.

The object of the present work was to establish a breeding laboratory culture of the British harvest mite, *Trombicula* (*Neotrombicula*) *autumnalis* Shaw, 1790. This was required in order to investigate the origins of the polymorphism observed in populations of larvae in nature.

The difficulty of feeding the postlarval stages of *T. autumnalis* hampered earlier attempts to rear this species (Hirst, 1915; André, 1920; Keay, 1937). Jones (1950) alone had some success, and was able to rear, with difficulty, a number of mites from the larval to the adult stage. This he accomplished by the 'forced feeding' of the nymphs on a mixture of yeast, molasses, agar and chicken faeces. Jones also tried, without success, to feed nymphs on moist, intact eggs of *Aëdes aegypti*. However, only when the shells of these were punctured, and the exuded contents placed close to their mouthparts, did the nymphs show any sign of feeding. Eggs of *Drosophila* sp., stripped of the outer shell, were also tried without success.

Jones (1950) reported that nymphs feeding on his 'mixture' took from 6–8 hr. to reach complete engorgement, and that much trouble was experienced in inducing the nymphs to feed for this period, since they habitually wandered away from the soft mass. To quote from Jones's own account: 'the patience required to make them feed amounted to careful individual nursing'. In no case did a nymph engorge itself when left in a cell with a quantity of food (Jones, 1951). Therefore a less laborious method of feeding the postlarval stages was sought, and trials were made with various non-living organic substances—singly or as mixtures; and also with various small arthropods and their eggs.

Early results of such trials were negative, but recently adult mites have been observed feeding on the eggs of an Entomobryid Collembolan, *Sinella curviseta* Brook.

METHODS

The mites used throughout these trials were collected from a site on the escarpment of the North Downs near Knockholt, Kent. Mites were obtained from this locality either as larvae (unfed or engorged) or adults.

Unfed larvae were collected by means of (1) a simple light-trap, modified from that of Jones (1950), or (2) from small white china tea-plates inverted on the soil, on to which larvae would walk, and on which they were easily seen and from which they were readily collected with a fine paint-brush. Unengorged larvae obtained from the field were then allowed to feed on baby rats or mice, housed in wire cages over a tray of water. Engorged larvae on leaving the host animal would then fall through the floor of the cage on to the surface of the water, where they remained floating on the surface film without harm, until picked up with a paint-brush and transferred to culture vials to undergo further development.

The above method resulted in a high mortality rate among unfed larvae, some being dislodged by the host, and others failing to engorge. In addition, the labour of collecting and feeding large numbers was considerable. For these reasons it was generally found more satisfactory to catch small rodents in Longworth traps. These animals were inspected and, if infested with larvae, were caged over trays of water, into which the larvae dropped after feeding was completed. The engorged larvae were then collected twice daily.

Normally, all the mites on the two common rodents (the field mouse, *Apodemus sylvaticus sylvaticus* Linnaeus, 1758, and the field vole, *Microtus agrestis hirtus* Bellamy, 1839) caught in the Longworth traps would have left the host by the fifth day after capture; the rodents were then usually released at the site of capture to 'collect' more larvae for subsequent recapture. The two rodents mentioned commonly harboured about 150 mites per animal at the height of the larval season.

Some wild rabbits (*Oryctolagus cuniculus* L.) were trapped alive by a friendly and interested gamekeeper; these were caged over water as before, and larvae collected. An average 'score' of larvae per rabbit, in the area in question, was about 500. One rabbit harboured over 900, but this seemed exceptional, so far as may be judged from the seven rabbits examined before the eradication of the local rabbit population by myxomatosis in the summer of 1954.

No evidence was found at Chevening to support the view that larval-over-wintering on vertebrate hosts is a common phenomenon in this species, as was suggested by the work of Elton & Keay (1936). Catches of small rodents over three winters failed to reveal a single larva after the first few autumnal frosts.

Occasionally guinea-pigs and golden hamsters (*Cricetus auratus*) were used as bait animals, but this method produced a 'catch' per animal per day of only about fifty mites, even if the cages were moved periodically to sample as large an area as possible; and it was therefore not suitable for obtaining large numbers for rearing.

Adult mites were obtained from the soil by a flotation method, first introduced by the earlier Japanese workers. The equipment used in the field consisted of a

sheet of waterproof tarpaulin which was supported on sticks, or merely laid in a natural depression in the ground. This produced a tank of convenient size, into which water was poured. The layer of turf in front of an old rabbit burrow was then removed and laid aside, and soil dug out from the hole with a shovel and sprinkled into the water. The surface of the water was then examined for the adult mites, yellowish in colour, about a millimetre long, and standing free on the surface film in a characteristic fashion. These were picked up with a brush, and the soil in the tank gently stirred to free any mites trapped by debris. The surface of the water was then re-examined before adding more soil. From fifteen to twenty adult mites were obtained in this way in 2–5 hr.

Culture methods for postlarval stages

The postlarval stages of Trombiculid mites are very sensitive to desiccation, and die rapidly if the relative humidity falls below 90 %. Therefore, in cultures, the artificial environment must be maintained continuously at, or very close to, saturation; but the presence of droplets of free water, in which the mites are liable to become entrapped and drown, must be avoided. The most satisfactory method of satisfying these rather exacting conditions has been found to be one based on that of Michener (1946), in which a dry mixture of plaster of Paris (or a proprietary dental plaster, prepared for dental modelling, such as 'Diocal') and approximately 10 % by weight of activated charcoal in powder form, is mixed with distilled water to form a thick paste which can be poured into culture tubes to a depth of about half an inch and allowed to set. The use of a 1:10,000 solution of 'Merthiolate' (sodium ethyl mercurithiosalicylate) in place of distilled water in solidifying the mixture was suggested by Sasa & Miura (1953); and this does to some extent control the prolific growths of fungi which are prone to develop under the conditions of high humidity prevailing in the culture tubes.

The function of the activated charcoal in the plaster mixture is twofold: first, to 'kill' the apparent toxicity of the plaster when used alone, and secondly to provide a convenient indication of the humidity within culture vessels, since the solidified mixture is almost black when wet, and becomes progressively lighter as it dries. This enables a ready check to be made, and a few drops of distilled water are added if required, enabling the layer of plaster to be kept continually moist, yet avoiding a surface film of free water. Such a mixture acts as a humidifier over long periods without attention, and absorbs or gives off water vapour as temperature conditions change.

Batches of about fifty fed larvae for rearing to the nymphal stage were usually put into 2 by 1¼ in. glass vials, with half an inch of the plaster mixture in the bottom. The vials were then closed with a cork stopper covered with thin polythene sheet (the latter prevented the mites from crawling into cracks in the cork, reduced evaporation through the pores of the cork itself, and prevented the growth of fungi on the inside face of the cork). Nymphal and adult mites were kept in screw-topped 4 oz. glass jars with a layer of plaster in the bottom.

The further development of the mites has been described fully elsewhere (Jones, 1951). About 20–30 days after the engorged larvae have fallen from the host, the

nymphs begin to emerge. Such nymphs will usually survive only about a week, and rarely longer than 3 weeks, without food; hence only a limited period is available for conducting feeding trials. Consequently, adult mites were obtained from the soil as described, since the adults are both larger and more robust than nymphs, are more amenable to life in captivity and will survive in culture tubes for up to 4 months under conditions of complete starvation. For these reasons, and since adults could be obtained from the soil throughout the year, whilst nymphs reared from larvae were normally only available from about August to November, it was found convenient to conduct most of the trials with the adult mites.

Non-living materials were placed close to a mite, or mites, in a 2 by 1¼ in. vial with a basal layer of plaster; the subsequent behaviour of the mites was watched under a binocular dissecting microscope, in subdued light, for an initial period of an hour. The tube would then be placed in a dark cupboard and re-examined periodically. This procedure was adopted because the adult mites, although eyeless, are apparently distressed by light and behave in an agitated manner, moving about the tube in a random fashion, ultimately tending to confine their activities to the darker side of the tube. It was thought probable that under such unnatural conditions of illumination, escape responses would supersede any possible responses to food.

In the case of arthropod eggs, and live and crushed arthropods, which could be counted and qualitatively assessed visually for signs of feeding, the procedure was to introduce a number of the organisms under test into the tube containing mites. The tube was then corked and placed in a dark place and examined daily over a period of about a week. A number of parallel trials were made on each occasion whenever possible, and the mites were also compared with starved controls for any possible increase in size. This provided a rough check of the visual assessment of the state of the test organisms made daily.

RESULTS

Nature of test substance or organism	Result of trials
Group 1 (non-living)	
(*a*) Fifteen combinations of hen egg yolk, albumen, cow's milk and glucose; together and singly	Consistently negative
(*b*) Grass roots, and other vegetable fragments obtained from soil at the same time as adult mites had been collected	Consistently negative
(*c*) Split sultana, banana slices, etc	Consistently negative
(*d*) Cheeses (various), liver sausage and meat pastes, etc.	Consistently negative
Group 2 (living or crushed arthropods or eggs)	
(*a*) Crustacea:	
Eggs and young of 'woodlouse' species	Negative
(*b*) Mollusca:	
Eggs and young of 'snail' species	Negative
(*c*) Myriapoda:	
Eggs and young instars of several undetermined species	Negative
(*d*) Hemiptera:	
Eggs and imagines of three species of aphid	Negative

Nature of test substance or organism	Result of trials
Group 2 (living or crushed arthropods or eggs)	
(*e*) Coleoptera:	
Eggs and larvae of *Callosobruchus* sp. and *Oryzaephilus surinamensis*	Negative
(*f*) Lepidoptera: Eggs and larvae of:	
Ephestia sp. *Tineola* sp. *Plodia* sp. *Paphia* sp. *Comma* Several undetermined species	Consistently negative
(*g*) Diptera: Eggs and larvae of:	
Musca sp. *Drosophila* sp. *Culex molestus* *Aedes aegypti* *Anopheles atroparvus* Various Dipterous larvae from fungi and soil	Consistently negative
(*h*) Collembola: Eggs and instars of:	
Isotoma spp.	Negative
Tomocerus sp.	Negative
Onychiurus sp.	Negative
Folsomia sp.	Negative
Sinella curviseta Brook	*Positive*
Instars only of:	
Miscellaneous species, obtained from soil taken from sites where adult mites were found, also other species from leaf-litter which failed to breed in the laboratory	Negative

Feeding trials with Sinella curviseta *Brook*

The use of various Collembola, and especially of *Sinella curviseta*, as a successful source of food for nymphal and adult Trombiculid mites was first described by Lipovsky (1951), who reported that twenty-five species of Trombiculid had thus been reared to the adult stage, of which fifteen species had laid eggs. In most cases it was the eggs of the insect that were attacked, but some species of Trombiculids were also found to eat the live insects, particularly those immobilized by moulting. Lipovsky also stated that other Collembola would serve as food, especially those of the family Entomobryidae, to which *Sinella* itself belongs. However, of many species of Entomobryid collected in the present study, none could be induced to lay eggs in the laboratory.

Ultimately, a colony of *S. curviseta* was obtained from the United States, through the courtesy of Lt.-Col. R. Traub, M.S.C., of the Walter Reed Army Medical Centre, Washington, where this insect was still maintained in culture by Mr L. J. Lipovsky. The insect is readily reared in the laboratory when provided with pellets of yeast as food, and lays eggs regularly. Preliminary trials with these eggs were, however, disappointing during the first few weeks after the arrival of the colony, since the adult mites to whom they were offered failed to feed. The trials were continued for a further period and it was found that the mites had begun to

feed on the *Sinella* eggs, and have now been observed in the act of piercing the egg shells and sucking out the contents.

The eggs when first placed in the tube containing the mites were ignored, but within half an hour or so the mite could be seen to approach an egg, explore it with the first pair of legs, moving round the egg whilst doing so. The first pair of legs were then used to roll the egg closer to the mouthparts, into a position such that the palps could be employed to prevent the egg from being forced away as the chelicerae were slowly driven through the egg membrane. The sucking out of the egg contents was then commenced. Whilst emptying the egg in this way, the hysterosoma was observed to pulsate slowly and occasionally to sway from side to side, being meanwhile raised well above its usual position. When the egg had been almost emptied of its contents, the chelicerae were withdrawn, the collapsed egg being at the same time pushed away by the straightening of the palps. The process as described above, from the initial puncture to the withdrawing of the chelicerae, occupied as little as 3½ min. During this time, the front legs were held clear of both egg and substrate, and often were gently waved to and fro. On one occasion a mite was observed to pierce an egg and suddenly become sluggish, movement ceasing entirely within a few seconds, appearing to all intents and purposes dead. However, about 10 min. later it again stirred, resumed normal feeding, rapidly ingested the contents of the egg and moved away.

Adults have been observed to feed on as many as four successive eggs within a few hours, although not all the eggs appeared to be entirely emptied. The time occupied in feeding on eggs subsequent to the first was usually longer, about 7 or 8 min.

Adult mites will consume eggs at a rate of approximately five or six a day.

On occasion, after leaving a punctured egg, adult mites were observed to resume the feeding posture, and make vigorous feeding movements—more vigorous in fact than those employed in feeding proper. This behaviour had been noticed before with *Trombicula autumnalis* in earlier negative trials with a variety of test organisms and substances, and appears to be a common feature of the behaviour of nymphal and adult Trombiculid mites in general. This behaviour has been referred to by Lipovsky (1954) as 'simulated feeding'. The process could also be construed as a method of drinking from the surface film of water, since it often occurs, though not exclusively, when free water is present in small cavities of the substrate.

By the use of the methods described, adult mites were kept alive for periods of over 8 months, in a healthy and active condition; *Sinella* eggs were supplied to the cultures at weekly or fortnightly intervals.

DISCUSSION AND CONCLUSIONS

The observations outlined above constitute the first record of a postlarval stage of *Trombicula autumnalis* feeding, without coercion, in the laboratory, on a type of diet that is available in the soil in which the active nymphs and adults are to be found. The major obstacle to the establishment of a breeding colony of *T. autumnalis* in the laboratory thus appears to have been overcome. The diet of nymphs and adult Trombiculid mites in nature is still uncertain, but the circumstantial

evidence for a natural diet of the eggs, or other immobile stages of small arthropods, seems overwhelming. It is of course possible that soil water containing suspensions of organic matter and bacteria may also form part of the food supply in nature, and the reports of the so-called 'simulated feeding' observed for a number of species may be connected with this possibility. Thus, although one of the primary obstacles to the establishment of a laboratory colony seemed at first sight to have been overcome, it became clear in the course of the ensuing months that this was only partly the case, since although the adult mites fed and flourished well on the diet of *Sinella* eggs, they did not lay eggs under any circumstances.

Thermal shocks and the like failed to stimulate oviposition, and the reason for this sterility is at present inexplicable, particularly since related species in the U.S.A. and elsewhere breed readily under apparently identical conditions, if supplied with the eggs of *Sinella*.

It is clear, then, that some unknown physiological requirement of *Trombicula autumnalis* remains unsatisfied in these circumstances.

Owing to the failure of the primary aims of these investigations, viz. to set up a breeding colony of the mite under controlled conditions, it has not proved possible to throw direct light on the problem of larval polymorphism. However, some incidental observations may be mentioned which bear on the question.

The site from which the mites were obtained is ecologically isolated from the surrounding area of the North Downs, and samples of larvae from this area were consistently morphologically uniform, corresponding in Richards (1950) classification to 'Normal type B'.

It is suggested that this ecological isolation may be a causal factor of the uniformity of larvae from Chevening, whereas samples of larvae from other parts of England and Europe frequently show much variation within and between samples, from time to time and from place to place.

For example, a number of larval samples sent to the writer by Dr L. Emmel, of Frankfurt-on-Main, Germany, showed polymorphic features in regard to the distribution and type of body setae; statistical treatment of the data indicated strongly the existence of two seasonal types of larvae. Of these, the 'early' form occurring from July onwards has short, single humeral setae, associated with short scutal and dorsal setae and nude tibial and galeal setae. By contrast the 'late' form, occurring in October–November, has long duplicated humeral setae, longer scutal and dorsal setae and ciliated tibial and galeal setae.

The distinction between these two types is not clear-cut and definite, such as to make it possible easily to assign any one larva to one group or the other, and certainly the distinction cannot reasonably be considered as more than of varietal rank at best. In my opinion, such findings, based on statistical analysis of measurements and other minutiae, should be treated with caution, but I feel they are suggestive of the occurrence of local biological races in this species of mite. Perhaps one might tentatively suggest that *T. autumnalis* in Britain and northern Europe is undergoing rapid local evolution and may in time, form a species-group, such as commonly occurs among the tropical members of the Trombiculidae. If, as I feel is reasonable, the situation found at Chevening Park may be regarded as

typical of such an area, I should add the proviso that populations in ecologically isolated and self-contained areas appear to remain phenotypically stable.

Concerning morphological variation among nymphs of this species of mite, such as Jones (1951) observed among individuals derived from batches of apparently identical larvae, I can only say that among nymphs derived from Chevening there is no evidence to confirm Jones's observations of long-haired and short-haired forms easily distinguishable by eye. Among the material from Chevening there does exist a certain degree of individual variation in size, body shape and length of body setae, but there is no evidence that this is other than of a purely random nature, particularly as malformed individuals are by no means rare at Chevening.

An interesting point arises in connexion with the advent of myxomatosis in my experimental area: prior to 1954, rabbits, occurring in very large numbers in the area, were undoubtedly the principal hosts of the larval mites, and adult mites could always be found in the soil at the burrow entrances.

The destruction of the rabbit population in 1954 was rapid and complete: within 3 weeks no trace of a rabbit remained, yet, up to 18 months later, adult mites could still be found in the soil in front of the now-deserted burrows in as great a number as before the destruction of the rabbits. I think that the most probable explanation for this, in addition to the possibility of adult survival for at least a year, is that pheasants, which are maintained at a high density at Chevening for the shooting, have superseded the rabbits as the principal larval hosts. Further, the pheasants can often be seen resting, out of sight until one approaches within a few feet, in the depressions which are numerous in front of the deserted warrens. Hence, if the birds spend a fair proportion of time in this situation, as they appear to do, engorged larvae will tend to accumulate in this situation in the same way as before.

Certainly, in the summer of 1955, larvae were as numerous as in previous years, and it is to be expected that this situation will continue in the future, in the absence of the rabbits, providing alternative hosts such as small rodents and ground feeding birds are present in sufficient numbers.

SUMMARY

1. Previous attempts to feed the postlarval stages of the British harvest mite, *Trombicula* (*Neotrombicula*) *autumnalis* Shaw, are outlined briefly.

2. Methods by which mites were collected from the field are described and discussed.

3. The methods adopted for providing a suitable artificial environment in the laboratory, and the method by which feeding trials were conducted are described. The materials and organisms supplied as possible sources of food are listed, together with the results obtained when these were offered to nymphs or adults in controlled trials.

4. The feeding behaviour of adult mites in response to the eggs of an Entomobryid Collembolan, *Sinella curviseta* Brook, is described.

5. The nature of the probable diet in nature is briefly discussed.

6. The possible influence of ecological isolation on the evolution of polymorphic larval types is discussed.

7. The effect on the mite population of the destruction of rabbits, the principal vertebrate host in the experimental area, by myxomatosis is outlined.

I wish to express my thanks to the following persons and institutions, without whose assistance this work would have been impossible. First, I should like to record my gratitude to the late Prof. P. A. Buxton, C.M.G., F.R.S., for the facilities provided in the Department of Entomology, London School of Hygiene and Tropical Medicine, and for his constant interest, sound advice, and many helpful criticisms made throughout this work; also to Dr D. S. Bertram for similar assistance. My thanks are also due to Colonel R. Traub, M.S.C., and Mr L. J. Lipovsky, of the Walter Reed Army Medical Center, Washington, U.S.A., and to D. A. Crossley, of Kansas University, for their joint courtesy in providing the colony of *Sinella* used in this investigation. Dr P. Armitage was kind enough to advise me on statistical questions. I am indebted to the Rt. Hon. Lord Stanhope, K.G., for his kindness in allowing me to collect material on his Chevening estate; also to his Head Keeper, Mr Chillcot, for his help and kindly interest. I wish to thank the University of London for a Postgraduate Studentship awarded to me for one year, and also the Medical Research Council for the award of a further Studentship after the expiry of the former.

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THE INTRANUCLEAR INCLUSIONS IN THE MID-GUT OF THE LARVA OF *ANOPHELES SUBPICTUS*

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INTRODUCTION

The object of this communication is to focus attention on certain interesting facts concerning the mid-gut of the larva of *Anopheles subpictus* previously reported in outline by us (Dasgupta & Ray, 1954, 1955). In the course of our routine examination on *Anopheles* larvae collected from different parts of the city and its outskirts, we directed our inquiry on certain histochemical problems. Our observation was mainly concentrated on detecting the sites of alkaline phosphatase activity in the mid-gut. Our interest, however, was quickly aroused by the occurrence in the secretory cells of the mid-gut of Feulgen-positive intra-nuclear inclusions. So far as we are aware, inclusion bodies of this nature have never been reported from Diptera.

MATERIAL AND METHOD

The batch of the larvae of *A. subpictus*, on which this communication is mainly based, was collected from a pool of rain water at Dhakuria, in the outskirts of Calcutta. Entire larvae and also decapitated larvae were fixed overnight in chilled 80% alcohol. They were later dehydrated in absolute alcohol, cleaned in benzol, embedded in 'tissuemat' and cut 8–10μ thick. The sections were subjected to Feulgen's nucleal reaction.

RESULTS

Feulgen preparations and the inclusion bodies

In normal secreting cells of the mid-gut, the nucleolus was surrounded by DNA granules and, even, sometimes was covered by DNA envelope. The nuclear membrane was Feulgen-positive and often Feulgen-positive material was found accumulated on the inner surface of the nuclear membrane. A number of DNA particles also occurred in the space which separated the nucleus from the nuclear membrane.

The epithelial cells which showed the intra-nuclear inclusion bodies were very conspicuous. A mature inclusion body appeared as a lump of DNA formed of innumerable globular bodies. Between a normal cell and one with a mature inclusion body there were found a series of transitional stages. In the earliest of the series the increase of DNA masses in the nucleus was noted. Later these masses appeared to coalesce and envelop the nucleolus. The lump of DNA formed as above began to grow bigger and bigger, and was separated from the nuclear membrane by a clear space. The mature inclusion body nearly obliterated the entire nucleus and the nuclear membrane had a crumpled appearance. Staining reactions suggested that the amount of DNA in the nucleus of other cells in the neighbourhood of the affected nucleus was reduced considerably.

It is to be noted that while the normal nucleus of the actively secreting cells of the mid-gut measured $5 \times 10\mu$, the one containing an advanced inclusion body measured $10 \times 14\mu$ along the broadest diameter.

DISCUSSION

It is difficult at present to assign a true significance to the inclusion bodies reported by us. However, we would like to draw attention in this connexion to the non-polyhedral disease, characterized by the hypertrophy of nucleus, reported in the fat cells of the larvae of cut-worm (*Euxoa segetum*) by Paillot (1936). Whether the inclusion bodies reported by us are the manifestation of a non-polyhedral disease remains to be seen.

Bedson (1954, personal communication) has kindly pointed out that the inclusion bodies reported by us looked like virus inclusions. Unlike many virus inclusions, however, these were Feulgen-positive, and so far as it is known, only the inclusions of the psittacosis-lymphogranuloma group of viruses and those produced by rickettsiae are positive with the Feulgen technique.

Further investigation of these inclusions had to be suspended because we could not again find the infected material.

We wish to record our thanks to Professor S. P. Bedson of the Bland-Sutton Institute of Pathology, Middlesex Hospital, London, for kindly examining our preparation of Feulgen-positive inclusion bodies and giving us his expert opinion on the subject.

Our thanks are due to Dr R. N. Chaudhuri, Director, School of Tropical Medicine, Calcutta, for his kind interest in the problem and the laboratory facilities accorded to us and to the Indian Council of Agricultural Research for the award of Research Fellowship to one of us (B.D.).

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ON A FILARIAL PARASITE, *DERAÏOPHORONEMA FREITASLENTI* N.SP., FROM THE GIANT ANTEATER, *MYRMECOPHAGA TRIDACTYLA* FROM BRITISH GUIANA, AND A PROPOSED RECLASSIFICATION OF *DIPETALONEMA* AND RELATED GENERA

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(With 9 Figures in the Text)

A number of filarial worms were collected from the mesentery of a giant anteater, *Myrmecophaga tridactyla*, which died in the London Zoo four months after its arrival.

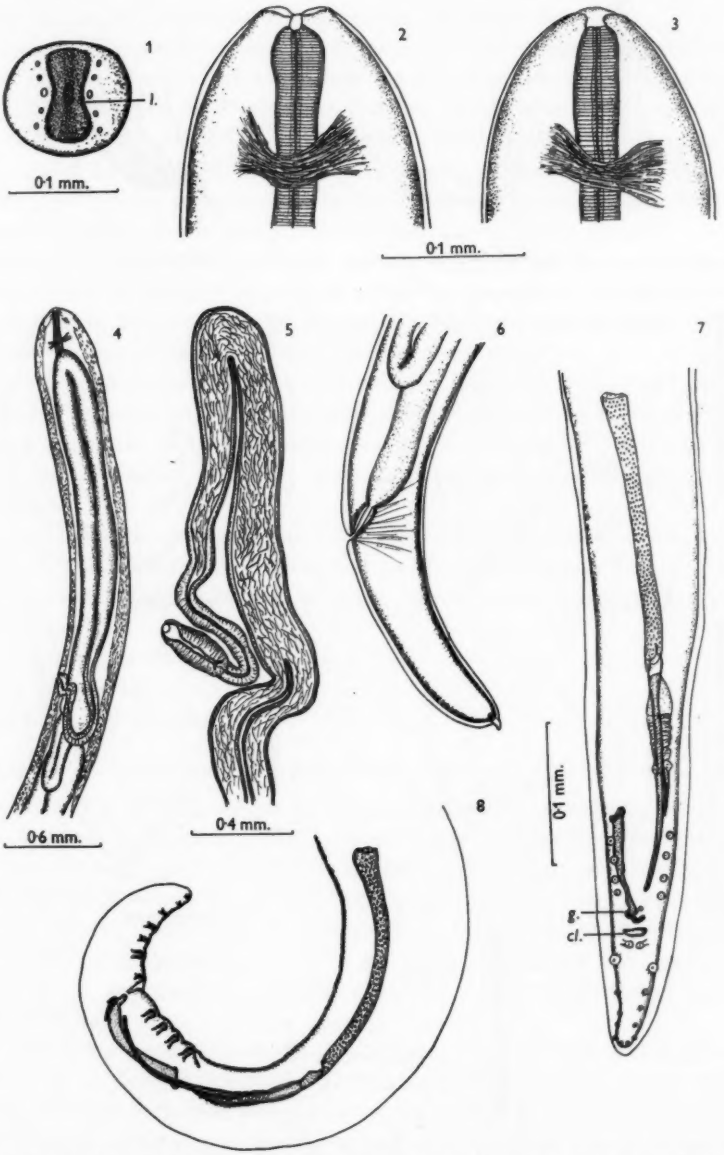
Genus *Deraïophoronema* Romanovitch, 1916

Deraïophoronema freitaslenti n.sp.

One complete male, two complete females and a number of pieces were available for study. These long worms are blunt anteriorly, but taper down gradually at their posterior ends in both sexes. The cuticle is smooth and devoid of any striations. The head-end has two rudimentary lateral lips, each with five papillae. There is a small buccal cavity. The mouth opening is oval, with the longer diameter dorso-ventral.

Female. The female is 62·0–68·2 mm. long and 0·33–0·36 mm. in maximum diameter. The oesophagus is 9·6–10·7 mm. long. The short anterior muscular part is 0·38–0·50 mm. The nerve ring is 0·21–0·25 mm. from the anterior end. The tail is 0·25–0·34 mm. long, and ends in a small single terminal spike. The non-protrusible vulva opens 2·1–2·4 mm. from the anterior end of the worm. The vagina and uterus may be directed immediately posteriad, or more usually, in the older specimens, it is directed as far anteriorly as the nerve ring before turning backwards when it then branches into two uteri. The anterior end of the vagina is more muscular and bulbous than the rest. The microfilaria is sheathless.

Male. The male is 37·5 mm. long and 0·27 mm. in maximum width. The oesophagus is 9·8 mm. long. The short anterior muscular portion is 0·42 mm. The nerve ring is 0·21 mm. from the anterior end. The cloaca opens 0·15 mm. from the posterior tip. The tail is provided with alae which are supported by four pairs of pre-cloacal papillae, six pairs of post-cloacal papillae, a single median pre-cloacal papilla, and a pair of papillae behind the cloaca, making a total of twenty-three papillae. The spicules are dissimilar and very unequal. The short, broad right spicule is distinctly curved and has a little notch before its tip. This spicule is 0·160 mm. long and 0·021 mm. wide. The long left spicule is 0·670 mm. It is pitted anteriorly and has a twist half-way down its length, and branches into two parts. The main part



Figs. 1–8. *Deraïophoronema freitaslenti* n.sp. Fig. 1. End-on view. Fig. 2. Ventral view of anterior end. Fig. 3. Lateral view of anterior end. Fig. 4. Anterior end of female. Fig. 5. Dissected view of vagina and uteri. Fig. 6. Tail of female. Fig. 7. View of male tail end. Fig. 8. Lateral view of male tail end. *cl.*, cloaca; *g.*, gubernaculum; *l.*, lateral lip.

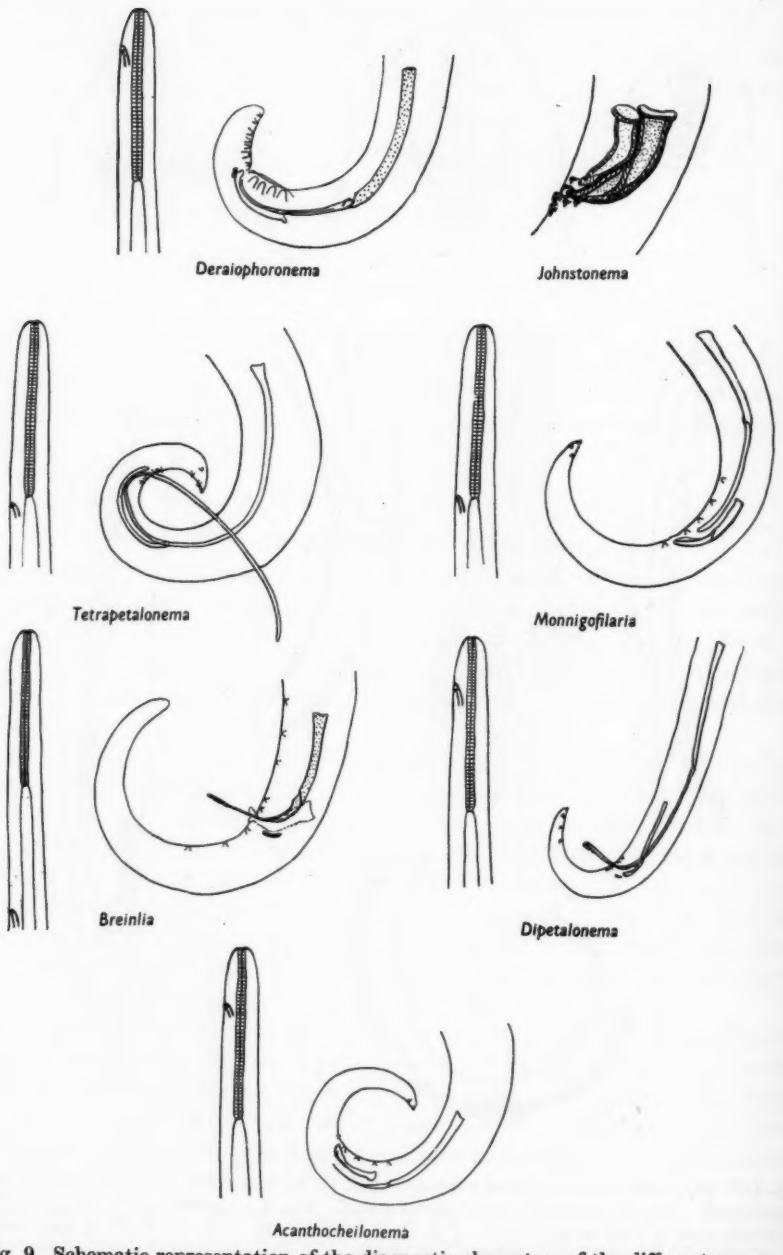


Fig. 9. Schematic representation of the diagnostic characters of the different genera.

remains fairly stout and solid and narrows down a little. The other part is thin and weakly chitinized and not easily seen, and appears rough and corrugated in the middle of its length. The small gubernaculum measures 0·038 mm.

Discussion. This genus appears not to have been recognized as a valid one until recently. It was regarded as a synonym of *Dipetalonema* by Yorke and Maplestone, 1926, of *Setaria* by Baylis and Daubney, 1926, and of ?*Dipetalonema* by Baylis, 1939. Chabaud (1952), in his review of the genus *Dipetalonema*, places *Filaria evansi* Lewis, 1882, under *Dipetalonema*. Later, however, Chabaud & Choquet (1953) rightly recognized the genus *Deraïophoronema* Romanovitch, 1916, and place *D. evansi* (Lewis, 1882) as genotype.

I believe this latter classification is correct, and do not have any hesitation in accepting the genus *Deraïophoronema* as valid. The genus as it is at the moment has only one species, the genotype, *D. evansi*. To this list must be added *D. spirale* (Molin, 1860) n.comb., and *D. freitaslenti* n.sp.

The new species is closely allied to *D. spirale* as described by Sandground (1938) and Lent & Freitas (1942). It is, however, easily distinguished by its much longer right spicule, which is three times the length of that given by Lent & Freitas, and by the presence of more pre- and post-cloacal papillae differently arranged.

The new species is named in honour of the two distinguished South American workers, Dr J. F. Teixeira de Freitas and Dr H. Lent.

Type. In the Helminthological Collection of the London School of Hygiene and Tropical Medicine.

Host. Myrmecophaga tridactyla.

Habitat. Mesentery.

Locality. British Guiana.

A proposed re-classification of Dipetalonema *Diesing*, 1861, *and related genera*

Chabaud (1952) revised the genus *Dipetalonema*, and considered as synonyms *Acanthocheilonema* Cobbold, 1870, *Tetrapetalonema* Faust, 1935, and *Loxodontofilaria* van den Berghe & Gillian, 1939. In a later paper, Chabaud & Choquet (1953) added to this list *Mönnigofilaria* Skrjabin & Schikhobalowa, 1948, and provisionally *Mansonella* Faust, 1926. Webber (1955), in a review of the *Dipetalonema* of primates, lists *Acanthocheilonema* as a synonym, agrees on the synonymy of *Tetrapetalonema*, and makes no mention of the other genera listed as synonyms by previous authors.

While studying *Deraïophoronema freitaslenti* n.sp., I have found the genus *Dipetalonema* as defined by both Chabaud and Webber to be unacceptable. According to their definition the genus contains some forty species with a most diverse collection of heterogeneous characters. Just to quote one or two examples: *D. annulipapillatum* Johnston & Mawson, 1938, has short, stout, subequal spicules in contrast to *D. perstans* in which they are slender and very unequal, the left one being several times longer than the right. Again, in *D. dracunculoides* the vulva is far forward, whereas in *D. blanci* it is at the junction of the oesophagus and intestine, and in *D. dasyuri* it is situated more posteriorly. The characters are thus

so diverse that the genus is obviously very unwieldy, and as defined by these authors is unsatisfactory.

In an attempt to throw some light on this confusion, I have rearranged the species under many new combinations. This was done by studying some of the *Dipetalonema* (*sic*) material at first hand, and depending for the rest on the descriptions and drawings, often inadequate, of the original and subsequent authors. Later, when some of the less well-known species are redescribed in finer detail, it may become necessary to remove some of them to other more suitable genera.

Species considered in this proposed reclassification belong to the genera *Acanthocheilonema* Cobbold, 1870, *Breinlia* Yorke & Maplestone, 1926, *Dipetalonema* Diesing, 1861, *Mönnigofilaria* Skrjabin & Schikhobalowa, 1948, *Deraïophoronema* Romanovitch, 1916, and *Tetrapetalonema* Faust, 1935. *Molinema* Freitas & Lent, 1939, is considered a synonym of *Acanthocheilonema*. Owing to inadequate information, *Loxodontofilaria* van den Berghe & Gillain, 1939, cannot be considered and must be regarded as *genus inquirenda*.

A new genus, *Johnstonema*, is erected for the reception of a species for Marsupials. It has a smooth cuticle, and short, stout, similar and subequal spicules.

Johnstonema n.gen. contains parasites of Marsupialia. They differ from all the other species in the group under discussion in that the spicules are short, stout, similar and subequal. The female has not been described. This genus has some similarity to *Onchocercella* Yorke & Maplestone, 1931, from Insectivora, especially in regard to the spicule, and in the arrangement of caudal papillae. The latter genus, however, has fusiform cuticular thickenings which places it in a different genus, whereas in *Johnstonema* the cuticle is smooth.

Tetrapetalonema contains parasites of Primates. Although they have two similar, slender and very unequal spicules as in *Dipetalonema*, the longer left spicule is simple and undivided, and unlike the other genera under discussion in which the spicule has two or more parts.

Deraïophoronema contains parasites of Ungulata and Edentata. It has its pre- and post-cloacal papillae in an almost continuous row, whereas there is marked discontinuity in the post-cloacal papillae in the other genera. The vulva opens far anteriorly.

Mönnigofilaria has been reported from Insectivora, Rodentia, Carnivora and Primates. When viewed ventrally, the left spicule is equal in width almost throughout its length, with no narrowing down distally as in the other genera. The vulva opens at the junction of the oesophagus and intestine.

Dipetalonema contains parasites of Primates. It is close to the above in spicular shape, but both spicules are long and slender and narrow down distally. The vulva opens far anteriorly.

Acanthocheilonema contains parasites of Edentata, Carnivora and Rodentia. The vulva opens far anteriorly, but the spicular shape is very different from that of *Dipetalonema*. The longer left spicule has a stout anterior part, a twist and then a rapid narrowing down of the medial part followed by a very slender distal part which is often undulated. The short right spicule is stout.

Breinlia contains parasites of Marsupialia. The left spicule has the stout proxima

part, a twist and a rapid narrowing down, but the distal third is a slender solid tube and may end in a slight enlargement. The distal end of the stout right spicule has a small distinct notch. The vulva is post-oesophageal.

Below is a key to the above genera, which were formerly considered as *Dipetalonema s.l.*

1. Male tail with pre- and post-cloacal papillae right to tip of tail in almost continuous rows. **Deraïophoronema** Romanovitch, 1916
— Male tail with pre- and post-cloacal papillae on tail disrupted soon after cloaca. 2
2. Spicules similar, stout and subequal. **Johnstonema** n.g.
— Spicules dissimilar and unequal. 3
3. Spicules simple and undivided. **Tetrapetalonema** Faust, 1935
— Spicules with two or more parts. 4
4. Vulva at junction of oesophagus and intestine, and distal half of spicule not very slender. **Mönnigofilaria** Skrjabin & Schikhobalowa, 1948
— Vulva not at junction of oesophagus and intestine, and at least distal third of left spicule is slender. 5
5. Vulva posterior to junction of oesophagus and intestine. **Breinlia** Yorke & Maplestone, 1926
— Vulva far forward of junction of oesophagus and intestine. 6
6. Right spicule long and slender, left spicule very long with distal half slender. **Dipetalonema** Diesing, 1861
— Right spicule stout and curved, left spicule with very slender and pointed distal third. **Acanthocheilonema** Cobbold, 1870 (syn. **Molinema** Freitas & Lent, 1939)

Acanthocheilonema Cobbold, 1870

(Syn. *Molinema* Freitas and Lent, 1939.)

Genotype: *A. dracunculoides* Cobbold, 1870. In Carnivora.

Other species:

A. anticlavum (Molin, 1858) n.comb. In Edentata.
Syn. *A. tatusi* Mazza & Anderson, 1926.
A. arbuta (Highby, 1943) n.comb. In Rodentia.
A. bifida (Molin, 1858). In Rodentia.
A. diacantha (Molin, 1858). In Rodentia.
A. finlayi Mazza & Fiora, 1932. In Rodentia.
A. multipapillata Walton, 1927. Host unknown.
A. procyonis (Price, 1955), n.comb. In Carnivora.
A. sprenti (Anderson, 1953) n.comb. In Rodentia.
A. travassosi (Artigas & Pacheco, 1933) n.comb. In Rodentia.
A. weissi Seurat, 1914. In Insectivora.

Breinlia Yorke & Maplestone, 1926

Genotype: *Breinlia trichosuri* (Breinl, 1911) Yorke & Maplestone, 1926. In Marsupialia.

Other species:

B. capilliformis (Baylis, 1934) n.comb. In Marsupialia.
B. dasyuri (Johnston & Mawson, 1938) n.comb. In Marsupialia.

B. dendrolagi Solomon, 1933. In Marsupialia.
B. johnstoni (Mackerras, 1954) n.comb. In Marsupialia.
B. robertsi (Johnston & Mawson, 1938) n.comb. In Marsupialia.
B. spelacea (Leidy, 1875) n.comb. In Marsupialia.
B. thylogali (Mackerras, 1954) n.comb. In Marsupialia.

Dipetalonema Diesing, 1861

Genotype: *Dipetalonema caudispina* (Molin, 1858) Diesing, 1861. In Primates.
Other species:
D. gracile (Rudolphi, 1809). In Primates.

Johnstonema n.gen.

Genotype: *Johnstonema annulipapillatum* (Johnston & Mawson, 1938) n.comb. In Marsupialia.
Other species:
Unknown.

Mönnigofilaria Skrjabin & Schikhobalowa, 1948

Genotype: *Mönnigofilaria setariosa* (Mönnig, 1926), Skrjabin & Schikhobalowa, 1948. In Carnivora.
Other species:
M. blanci (Chabaud, 1952) n.comb. In Rodentia.
M. digitata (Chandler, 1929) n.comb. In Primates.
M. rodhaini (Peel & Chardome, 1947) n.comb. In Primates.
M. streptocerca (Macfie & Corson, 1922) n.comb. In Primates.
M. sunci (Sandground, 1933) n.comb. In Insectivora.

Deraïophoronema Romanovitch, 1916

Genotype: *Deraïophoronema evansi* (Lewis, 1882). In Ungulata.
Syn. *D. cameli* Romanovitch, 1916.
Other species:
D. freitaslenti n.sp. In Edentata.
D. spirale (Molin, 1860) n.comb. In Edentata.

Tetrapetalonema Faust, 1935

Genotype: *Tetrapetalonema marmosetae* Faust, 1935. In Primates.
Other species:
T. atelensis McCoy, 1936. In Primates.
T. parvum McCoy, 1936. In Primates.
T. perstans (Manson, 1891) n.comb. In Primates.
T. vanhoofi (Peel & Chardome, 1946) n.comb. In Primates.

As some of the undermentioned species were inadequately described and illustrated, and as the males of many of these species remain undescribed, their generic position cannot be determined.

Genera inquirenda

Tetrapetalonema berghei Chardome & Peel, 1951 (females only).
Filaria grassii Noè, 1907.
F. hippopotami Leiper, 1910.
Loxodontofilaria loxodontis van den Berghe & Gillain, 1939.
Filaria nicollei Mazza, 1930.
Dipetalonema okapiae Fain, 1948.
D. petteri Chabaud & Choquet, 1955 (females only).
Skrjabinofilaria pricei Vaz and Pereira, 1934.
Dipetalonema rarum Johnston and Mawson, 1938.
Filaria recondita Grassi, 1889.
Dipetalonema tenue Johnston & Mawson, 1938.

Species such as *Filaria roemeri* Linstow, 1905, *F. websteri* Cobbold, 1879, etc., which have already been removed from *Dipetalonema s.l.*, have been omitted from the list.

SUMMARY

1. A description is given of a new filarial parasite, *Deraïophoronema freitaslenti* n.sp. from the mesentery of the giant anteater, *Myrmecophaga tridactyla*, from British Guiana.

2. A proposed reclassification of *Dipetalonema* and related genera is given. Genera considered as valid are *Acanthocheilonema*, *Breinlia*, *Dipetalonema*, *Mönnigofilaria*, *Deraïophoronema* and *Tetrapetalonema*. *Molinema* is considered to be a synonym of *Acanthocheilonema*.

3. A new genus, *Johnstonema*, is erected for the reception of *J. annulipapillatum* (Johnston & Mawson, 1938).

I wish to express my gratitude to Professor J. J. C. Buckley for his interest in the progress of the work, the Zoological Society of London for the material, Professor J. Fraga de Azevedo, Director of the Instituto de Medicina Tropical, Lisboa, Portugal, for kindly sending a pair of *Dipetalonema dracunculoides*, and to Patricia M. Thomas (neé Mawson) who so very kindly re-examined the types of *J. annulipapillatum* and gave me the necessary information I required.

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HARMOSTICANA GARNHAMI GEN.NOV., SP.N. (LYGAEIDAE: HEMIPTERA-HETEROPTERA) ASSOCIATED WITH A MAMMAL IN EAST AFRICA

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(With 1 Figure in the Text)

The genus and species of Lygaeidae described and figured herein was obtained with several others from a squirrel's nest at Kodera, S. Nyanza Province, Kenya, East Africa, by Dr Garnham, Professor of Medical Protozoology at the London School of Hygiene and Tropical Medicine.

Dr Garnham's description of the animal occupying the nest, however, suggests that it was not a squirrel, but that it might be *Grammomys* (*Dolichurus*) *surdaster* Thomas & Wroughton 1908, which had taken over an abandoned squirrel's nest.

The new genus to which I give the name *Harmosticana* is closely allied to *Harmostica* (Distant) in which the sole species—*ornata*—was collected in Ceylon. The type of this species is in the British Museum (N.H.), London.

In the British Museum also are specimens belonging to *Harmostica*, but these may possibly represent a new species. They were found in Madras and other parts of Southern India, those from Madras having been discovered in nests of the squirrel *Funambulus palmarum* (Linnaeus, 1766).

The genus *Harmostica* was erected by Bergroth in 1918, to replace *Edulica* Distant which had already been used by Hampson.

Attention is drawn to the fact that Bergroth (1918) also wrote this name as *Harmosticta*.

Harmosticana gen. nov.

Size small. Antennae moderately thick; basal segment extending beyond apex of head. Ocelli widely separated. Rostrum extending to posterior coxae; segment I extending beyond base of head, subequal in length to segment 2. Pronotum transverse, wider than long; anterior margin strongly concave. Scutellum triangular, acute apically; disk feebly depressed. Hemelytra extending to apex of abdomen. Anterior femora incrassate; median and posterior femora moderately incrassate.

Type species, Harmosticana garnhami *sp.n.* (Fig. 1)

Colour. Segments 1 and 2 of antennae light brown; segment 2 suffused with piceous basally; segments 3 and 4 piceous. Rostrum testaceous. Head and body (except scutellum) light brown; pronotum posteriorly, meso- and metapleura suffused with piceous. Clavus, area between claval suture and *Cu*, base of area between *Cu* and *M*, pale testaceous; apex of clavus with dark brown suffusion; remainder of corium piceous with costal margin very narrowly brown;

membrane infumate with piceous suffusion basally. Scutellum piceous. Legs pale testaceous.

Structure. Antennae sparsely setose. Postocular with very few, moderately long, erect setae. Pronotum anteriorly with a transverse arcuate sulcus and row of punctures; anteriorly laterally with a few very short backwardly directed setae;

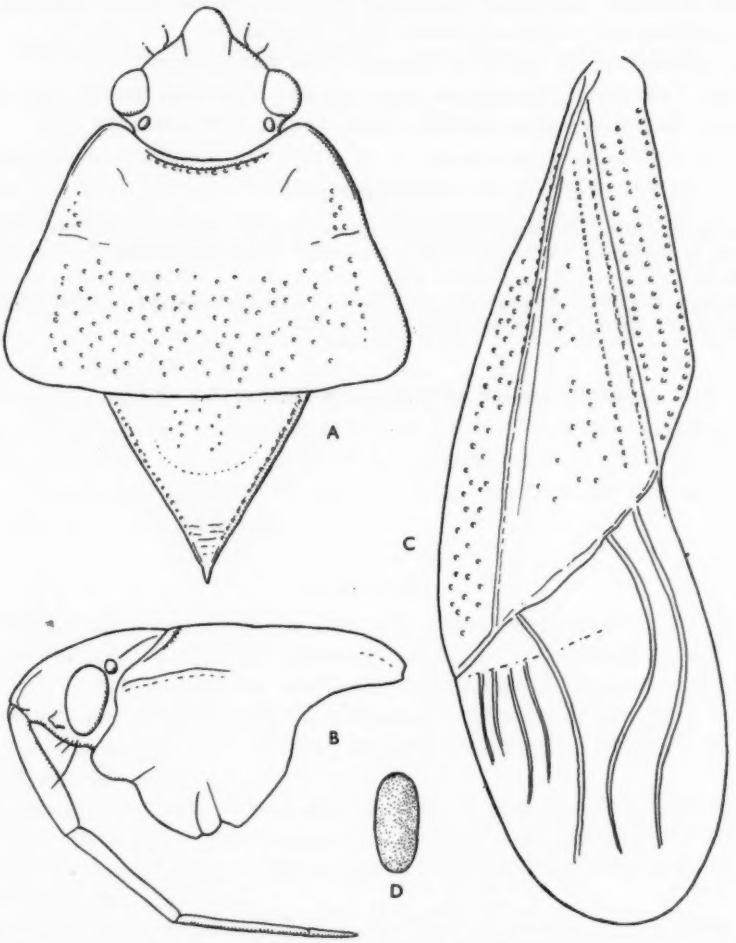

Fig. 1. *Harmosticana garnhami* gen. nov., sp.n. A, head, pronotum and scutellum, (dorsal view); B, head and pronotum (lateral view); C, hemelytron; D, ovum.

lateral margins narrowly dorso-ventrally compressed in anterior half; posterior margin almost straight. Pronotum with widely separated, small punctures. Scutellum with a very few small punctures medially and larger punctures laterally.

Total length, 4·50 mm.; hemelytra, 3·30 mm.; greatest pronotal width, 1·60 mm.

1 ♀ (holotype), Kenya, S. Nyanza Province, Kodera, 20 January 1956, P. C. C. Garnham. Holotype in British Museum (N.H.), London.

Somewhat similar in coloration to *Harmostica ornata*. It differs from *Harmostica* in having segment 2 of the antennae less than twice as long as segment 1, segment 4 considerably longer than segment 3, the ocelli less widely separated, the interspace being less wide than the vertex between the eyes, all segments of the rostrum relatively thick, segment 1 extending beyond the base of the head and subequal in length to segment 2, segment 3 shorter than segment 2 and the pronotum, scutellum and corium somewhat feebly punctate.

Four almost mature ova were dissected from the specimen.

Ovum. Cylindrical, rounded at each end and somewhat narrower at one end. Glabrous. Yellowish with a metallic lustre. Length 1·00 mm. (Fig. 1*D*).

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DEVELOPMENT OF *PARAMPHISTOMUM SUKARI* DINNIK, 1954 (TREMATODA: PARAMPHISTOMIDAE) IN A SNAIL HOST

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(With 9 Figures in the Text)

Since the publication of the description of *Paramphistomum sukari* Dinnik, 1954, from cattle in the vicinity of Nairobi, Kenya, a survey has shown that this paramphistome is widespread in Kenya, where *P. sukari* has been found in the reticulum of native cattle in the Kikuyu and Masai Reserves and Northern Frontier Province and also in grade cattle on European farms. This species also occurs in Uganda, where it was recovered from the reticulum of cattle in Ankole and in cattle from Karamoja, when the latter were slaughtered and examined at the Municipal Abattoir in Kampala, Uganda.

In Kenya, *Biomphalaria pfeifferi* was found to be an intermediate snail host of *Paramphistomum sukari* and rediae as well as free and encysted cercariae of the species have been described (Dinnik, 1954). The present observations were carried out to study the development of *P. sukari* and, in particular, the succession of its redial generations in a snail host.

MATERIAL

To obtain initial material for the following observation, about 2000 encysted cercariae emitted by a naturally infected specimen of *Biomphalaria pfeifferi* were fed to a calf. When the calf was sacrificed 85 days later 734 mature specimens of *Paramphistomum sukari* Dinnik were recovered from the reticulum. Eggs collected from these trematodes were hatched and miracidia used in attempts to infect laboratory-reared snails.

Laboratory-bred snails, *Biomphalaria pfeifferi nairobiensis* (Dautzenberg) and *B. sudanica tanganikana* (Bourguignat)* were exposed to miracidia of *Paramphistomum sukari* but only *Biomphalaria pfeifferi* became infected with larval stages of this stomach fluke.

During the time of the observations the snails were kept at a temperature varying between 24° and 27° C. and periodically from one to four snails were dissected in order to follow the larval development of *Paramphistomum sukari*.

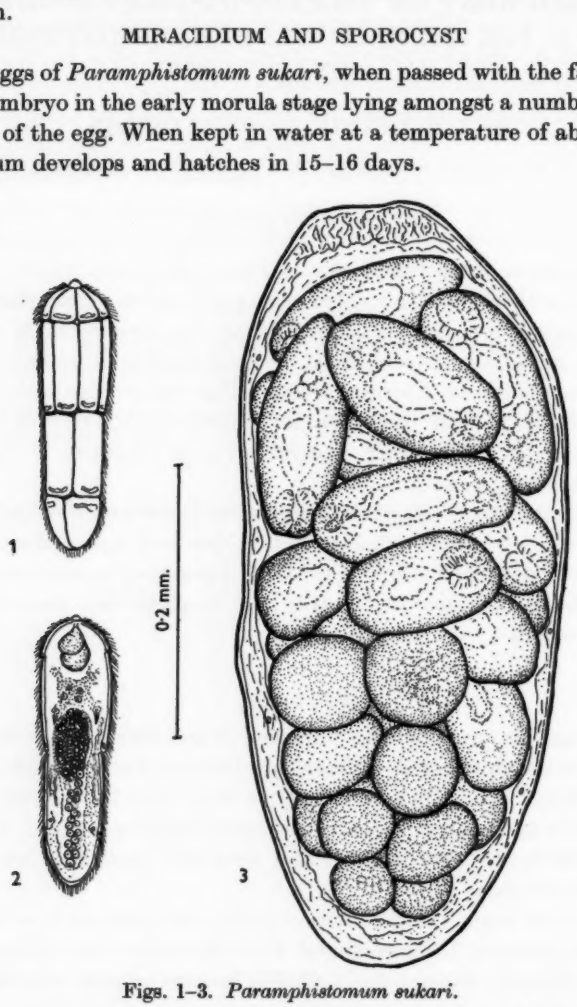
About 585 encysted cercariae obtained from the experimentally infected specimens of *Biomphalaria pfeifferi* were given to a calf to confirm the purity of the material and the identity of the species developing in the snails. The calf started

* The authors are indebted to Dr G. Mandahl-Barth of Danmarks Akvarium, Copenhagen, for the identification of the snails used in our experiments. The parent wild snails originated from Sukari Dam at Nairobi and from a pond at Kusa, Kavirondo, Kenya.

passing paramphistome eggs on the 79th day and, when it was sacrificed on the 162nd day, 365 mature specimens of *Paramphistomum sukari* were recovered from the reticulum.

MIRACIDIUM AND SPOROCYST

Each of the eggs of *Paramphistomum sukari*, when passed with the faeces of cattle, contains an embryo in the early morula stage lying amongst a number of yolk cells in the centre of the egg. When kept in water at a temperature of about 26–27° C., the miracidium develops and hatches in 15–16 days.



Figs. 1–3. *Paramphistomum sukari.*

Fig. 1. Miracidium, showing the arrangement of the epithelial cells. Fig. 2. Germinal material in a miracidium. Fig. 3. Sporocyst.

The miracidium is from 202 to 238 μ long and 49 to 59 μ wide and is covered with twenty ciliated epithelial cells arranged in four transverse rows of 6, 8, 4 and 2 cells per row respectively (Fig. 1). The germinal material in the miracidium is situated in the middle of the body cavity posterior to the pair of flame cells. It consists of from twenty to thirty germinal cells closely packed together in a compact morula-like group. The cells are somewhat irregular in shape, and each has a large nucleus surrounded by a thin layer of cytoplasm. The germinal cells vary in size from 10 to

15μ. A number of round cells with large nuclei are present in the posterior part of the body cavity of the miracidium. These cells extend from the posterior end of the body cavity alongside both its dorsal and ventral sides to the morula-like group of germinal cells (Fig. 2).

In an attempt to infect snails, they were exposed to a number of miracidia which quickly concentrated round and entered them during the first hour. The first specimen of *Biomphalaria pfeifferi* was dissected 12 days after exposure to miracidia and eleven sporocysts were found which, by that time, had grown into oval bodies measuring 0·53–0·71 by 0·23–0·26 mm. and containing from 4 to 10 small developing rediae and from 10 to 15 embryo balls in their body cavities. Sporocysts were recovered from the infected snails up to the 19th day after their exposure to miracidiae. They were broadly oval in shape, measuring up to 0·84 mm. in length by 0·41 mm. in breadth, and only a few of them had a shallow constriction round the middle of the body. No birth pore was seen in the sporocyst (Fig. 3). As a rule sporocysts were found to be localized in the mantle and the tissue of the respiratory cavity of the snail but in two snails, besides several sporocysts found in the tissue of the mantle and respiratory cavity, one and two sporocysts respectively were recovered from the liver.

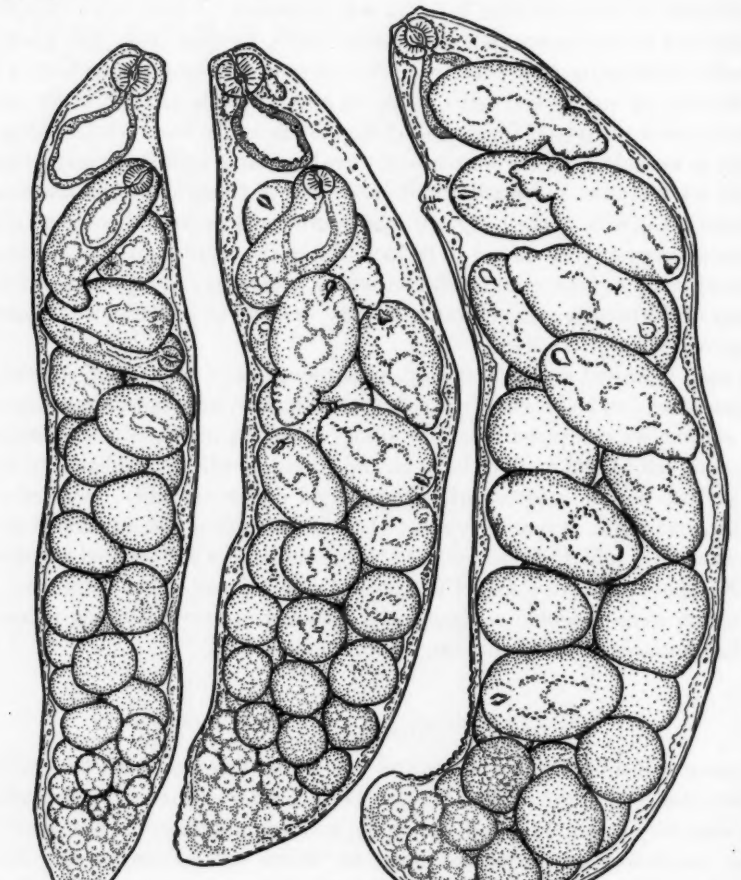
In a snail dissected on the 19th day, a sporocyst was found which showed signs of degeneration; its body was dirty yellowish in colour, less transparent than those of the sporocysts recovered from the snails sectioned previously, and contained a few young rediae and embryo balls but no germinal cells. In addition to this old sporocyst 265 rediae, some of which contained daughter rediae, were recovered from the same snail. Apparently most of these 265 rediae had developed in other sporocysts which had died and perished before the snail was dissected for examination. No sporocysts were found in another snail dissected the same day, or in snails which were dissected subsequently, although numerous young rediae were found in the tissue of the mantle and respiratory cavity.

REDIAE

Free young rediae of the first generation which had emerged from the sporocysts were first observed in the snails dissected 14 days after exposure to miracidia. The rediae were very small, 0·18–0·35 mm. long, and were found in the tissue surrounding the respiratory cavity of the snail, i.e. where the sporocysts are normally found. In another snail, which was dissected 2 days later, many young rediae were found migrating from the tissue of the respiratory cavity to the liver and eight young rediae were recovered from the liver itself. The rediae recovered from the liver were 0·51–0·58 mm. long and already contained up to 20 embryo balls in their body cavities.

The first rediae with daughter rediae and redial embryos developing in the body cavity were recovered from the liver of a snail dissected on the 19th day after exposure to miracidia. By that time the rediae were 0·57–0·79 mm. in length, their pharynxes varied from 0·039 to 0·043 mm. in diameter and their guts were 0·07–0·12 mm. long. Three pairs of flame cells, one situated anteriorly, the second

in the middle and the third in the posterior part of the body, were clearly seen in each redia. Each parent redia contained a germinal mass and cells at the posterior end and from 27 to 48 embryo balls tightly packed in the middle part of the body cavity. The size of the balls gradually increased towards the anterior part of the body cavity where from one to five developing daughter rediae could be recognized (Fig. 4).



Figs. 4–6. *Paramphistomum sukari.*

Fig. 4. First-generation redia during the phase of redial production. Fig. 5. First-generation redia changing from redial to cercarial production. Fig. 6. First-generation redia during the phase of cercarial production.

The first rediae of the second generation were observed free in the liver tissue of a snail dissected 21 days after infection. No morphological difference, except their smaller size (length 0·13–0·20 mm.) was found between the young rediae which

emerged from the parent rediae of the first generation and those which developed in the sporocysts.

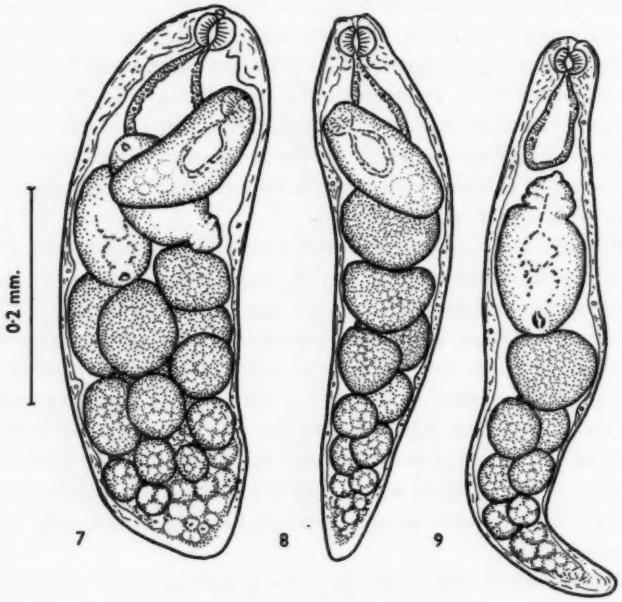
In snails dissected on the 23rd day after infection, daughter rediae and cercariae were found developing in the rediae of the first generation. These parent rediae were slightly larger, 0·73–0·88 mm. long, than those producing daughter rediae only. They usually contained one or two daughter rediae in the anterior part of the body cavity, one to three cercarial embryos with short rudimentary tails, several recognizable cercarial embryos without tails and from 25 to 43 embryo balls which decreased in size towards the posterior end of the body cavity where masses of germinal cells were seen. The embryo balls, developing cercariae, and young daughter rediae are so closely packed that even the parent redia's energetic movements cannot disturb their position in the body cavity. Thus the arrangement of the embryos in the body cavity of the parent redia not only showed clearly the successive phases of embryonic development of either daughter rediae or cercariae, but also left no doubt that in the first-generation rediae the production of cercariae followed that of daughter rediae (Fig. 5).

About half of the large rediae recovered from the snails dissected 23 days after exposure to miracidia contained only developing cercariae and many embryo balls. Because these rediae were somewhat larger than those containing both redial and cercarial embryos, and much larger than the rediae producing daughter rediae only, we considered them to be first-generation rediae which had already passed the first phase of their life, during which they produced only daughter rediae and had entered into the phase of cercarial production. No free cercariae were seen in the snail on the 23rd day of larval development. Many small young rediae, apparently rediae of the second generation, were observed in the snails. None of these young rediae contained recognizable redial or cercarial embryos, but only a few embryo balls and germinal cells.

In a snail dissected on the 25th day, the following larval stages were found: rediae of the first generation producing cercariae; free cercariae, which had escaped from the first-generation rediae and continued their development in the snail tissue, and many rediae of the second generation ranging from very small and young ones to rediae which contained redial embryos in their body cavities. In the snails dissected on the 27th and 28th days many second-generation rediae were found, containing both daughter rediae and cercariae developing together in their body cavities. The first-generation rediae found in these snails were at the peak of cercarial productivity. These rediae contained from one to five developing cercariae with short tails in the anterior part of the body cavity, from ten to fifteen tailless cercarial embryos in the middle and as many as 30 embryo balls and masses of embryonal or germinal cells in the posterior part of the body cavity. At this stage the first-generation rediae measured from 0·95 to 1·81 mm. in length (Fig. 6). The second-generation rediae were readily distinguished from those of the first generation by their smaller size and smaller number of embryos in their body cavities. The length of the second-generation rediae containing redial or cercarial embryos varied from 0·46 to 0·54 mm. More than two daughter rediae or three developing cercariae with rudimentary tails were never seen in the body cavities of

these rediae and the number of embryo balls did not exceed fifteen and was usually found to be less than ten in each redia (Fig. 7).

A few old rediae were observed first in a snail dissected on the 33rd day and almost all the first-generation rediae found in a snail dissected 35 days after exposure to miracidia were old and in the last stage of their life. The old rediae possessed no germinal masses or cells in the posterior end of the body as these had been completely exhausted by this time. They contained a small number of embryo



Figs. 7–9. *Paramphistomum sukari*

Fig. 7. Second-generation redia containing a daughter redia and a cercaria at the same time. Figs. 8, 9. Rediae probably belonging to the third generation recovered from a snail 35 days after its exposure to miracidia.

balls floating loosely in the body cavity and a few developing cercariae or rediae; sometimes both cercariae and rediae were present in the same parent redia. The old rediae were dirty greyish in colour, less transparent and, because they contained a smaller number of embryos than the rediae at the peak of their period of productivity, their bodies were contracted and transversely wrinkled.

The second-generation rediae found in the snail dissected on the 33rd day were principally producing cercariae, but one redia, which was 0·64 mm. long and contained one cercaria, four daughter rediae, and 12 embryo balls in its body cavity, was seen.

The snail dissected on the 35th day was infested with several hundred rediae and numerous cercariae developing free in the tissue of the liver. Besides the first- and second-generation rediae there were very many young rediae, about one-third

of the total number of rediae found in the snail, which were 0·50–0·55 mm. long and contained a few embryo balls and either one cercarial or redial embryo in the body cavities (Figs. 8, 9). It is probable that these small rediae with a few embryos inside were the third-generation rediae whose growth was impeded by overcrowding of the snail with numerous rediae and cercariae all developing in the liver at the same time.

In the last snail of the series, dissected 53 days after exposure to miracidia, there were no large rediae seen which could be identified as belonging to the first generation. It appeared that the first-generation rediae had completed their life span and died by that time. Old rediae recovered from the snail were smaller than those of the first generation observed in the snails dissected on the 35th day. Some of these old rediae contained cercariae and daughter rediae, together with a few embryo balls in their body cavities. Many mature rediae found in the snail were full of developing cercariae and some young rediae contained daughter rediae, whilst most young rediae had only embryo balls in their body cavities. Up to the 53rd day of larval development the progeny of the three or four successive generations of rediae were overlapped so that it was impossible to distinguish them from one another with certainty. It was, however, evident that as in *Paramphistomum microbothrium*, whose life-history has been described by the authors (1954), the infection of *P. sukari* in a snail host was maintained by a succession of redial generations.

CERCARIAE

As in the other species of Paramphistomidae whose cercarial development is known, cercariae of *P. sukari* leave their parent rediae when still immature and continue their development to maturity in the tissue of the snail host. In this experimental series, cercariae which had emerged from their parent rediae were first observed 25 days after exposure of the snails to miracidia. They were cercariae which had developed in the rediae of the first generation. Cercariae leaving parent rediae of the second generation were first observed 30 days after exposure of the snail to miracidia, and snails dissected thereafter were found to be harbouring numerous cercariae of different generations and in various stages of development towards maturity.

The body length of newly born cercariae varied from 0·12 to 0·16 mm., and the broad rudimentary tails of the cercariae were about half of the body length. The oral sucker in a newly born cercaria was well developed and clearly seen, with the mouth encircled by oral papillae, but the acetabulum was almost unrecognizable. After leaving the parent redia, the cercaria grows in size, feeding on the liver tissue of the snail. The eye-spots become prominent shortly after its emergence from the parent redia. When the young cercaria has almost reached the size of the mature cercaria and the acetabulum becomes easily visible, pigment starts to grow out from the eye-spots and spreads in irregular dendritic branches over the whole surface of the body. At this stage the excretory ducts are clearly visible, although they are not yet filled with granules. Two main excretory ducts join the excretory bladder at the acetabulum and from the bladder the ducts pass forwards and

outwards and then turn inwards. In the middle of the body the two main ducts are connected by an arch-shaped anastomosis lying across the body and a short median diverticulum is present running forwards from the centre of this anastomosis. In front of the anastomosis the main excretory ducts turn outwards and then inwards again posterior to the eye-spots; they pass forwards ventrally past the eye-spots and reach the sides of the oral sucker. No diverticula were observed arising from the distal parts of the main ducts in the region of the eye-spots.

When the cercaria approaches maturity the granules and rods of the cystogenous cells increase in number so that when combined with the heavy pigmentation, they make the body opaque and obscure the internal structure of the cercaria.

Mature cercariae began to emerge from the infected snails 35 days after their exposure to miracidia. Thus it appeared that the cercariae required about 10 days after leaving the parent redia to develop to maturity.

The mature cercariae of *P. sukari* are heavily pigmented, show two dark eye-spots and are about 0·25 mm. long and 0·17 mm. broad, the tail being about twice the length of the body. After a short swimming period the cercariae encyst and the diameter of the cysts is from 0·127 to 0·138 mm.

SUMMARY

The development of *Paramphistomum sukari* Dinnik in a snail host is described with the emphasis laid on the succession of redial generation.

The sporocyst gives birth to about twenty to thirty rediae. These rediae of the first generation commence with the production of daughter rediae then enter the second phase of their productivity during which they produce cercariae. The daughter rediae, or the rediae of the second generation, repeat these two phases during their lives, commencing with redial production and after that changing to the production to cercariae. Both the first- and second-generation rediae are able to produce a few daughter rediae at the end of their life. There is evidence that the subsequent generations of rediae are also able to give birth to daughter rediae and cercariae.

As a result the successive generations of rediae maintain the infection in an intermediate host for a long time, probably as long as the infected snail can survive.

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A CHECKLIST OF TROMBICULID MITES OF THE ORIENTAL AND AUSTRALASIAN REGIONS

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(With 26 Figures in the Text)

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INTRODUCTION

Four major works on trombiculid mites were published in 1952, and a fifth, a checklist completed up to that year, was published in 1954. The monograph by Womersley (March 1952) described 77 new species, redescribed the larvae of some 177 more, and described or redescribed post-larval stages of 53 species. Except for the post-larval stages, it was confined to species of the Asiatic-Pacific area. Some additions to this monograph, and a list of species with addenda, corrigenda, and taxonomic rearrangement, were published recently (Womersley & Audy, 1957). Wharton, aided by Fuller (1952), included a critical checklist of world species (443 names), but of course excluding Womersley's many new species (which were simply listed in an Appendix). Fuller (December 1952) published a detailed and fully documented study of trombiculids (46 species) in the Oudemans collection in Leiden. Gunther (July 1952) published a well-documented checklist of trombiculids of Asia and Australia, but this is superseded by the list of Wharton & Fuller, which is at present the most authoritative and complete checklist available. Radford (1954, submitted in February 1953) published an abbreviated checklist, apparently following Womersley but with some taxonomic rearrangement, omitting documentation and listing some but not all host records. Except for Radford's inclusion of Womersley's species, and Wharton & Fuller's cross-references to Fuller, none of these works contains references to the others, while they all adopt somewhat different taxonomic arrangements, so that some confusion prevails. Since that time, furthermore, some 23 genera and subgenera have been raised and a number of new species described, while a better understanding of

taxonomic relationships has been gained by studies of material in Malaya, in Africa (largely by P. H. Vercammen-Grandjean), and in the U.S.A. An undocumented checklist of 85 Malayan species has been published elsewhere (Audy, 1956*d*), together with details of naked-eye appearances, favoured sites of attachment, and principal hosts.

The present checklist has been prepared to link all these works together, to bring the taxonomic arrangement up to date, and to add data and species published since 1952. In order to shorten this paper, the checklist of Wharton & Fuller (1952: 40–104) is taken as a basis and only additional data given.

Exclusively palaearctic species, including the many species now known from Japan and Korea, are outside the scope of this checklist. The Japanese species have recently been listed, and host and faunal lists published, by Suyemoto & Toshioka (1955). Other important references to mites from Japan, Korea and the U.S.S.R. are Sasa (1953), Sasa & Jameson (1954), Jameson & Toshioka (1954) and Schluger (1948).

TAXONOMIC ARRANGEMENT

The terminology and taxonomic arrangement in the present paper is based on that of Wharton & Fuller, but takes account of revisions and additions by Audy (1954*b*, 1956*a–d*), Audy & Domrow (1957), Audy & Nadchatram (1957*a*, *b*), Audy & Womersley (1957), Domrow (1957*a*, *b*) and Vercammen-Grandjean & Audy (1957), as well as by the authors of new genera and subgenera listed below. A number of characters of taxonomic importance are illustrated in the accompanying figures. The taxonomy of trombiculids is largely based on characters of the parasitic larvae or chiggers.*

The list which follows gives the genera and subgenera adopted by the present writer. It will be noted, on comparing this arrangement with that of Wharton & Fuller, that *Blankaartia* [= *Tragardhula*] (see p. 245 and Womersley & Audy, 1957), *Eutrombicula*, *Fonsecia* and *Neotrombicula* are here treated as genera and not as subgenera of *Trombicula*; *Sauriscus* is not a synonym of *Tecomatlana*; *Euschongastia* and *Ascoschongastia* are divided into subgenera; *Walchia*, *Schongastiella* and *Gahrliepia* (= *Gateria*) are treated as subgenera of *Gahrliepia* following Womersley (1952), Traub & Evans (1954, 1957), and Traub & Morrow (1955); *Walchiella* and *Pseudoschongastia* are considered to be definitely Trombiculine (not Gahrliepiine = Walchiine), the former being treated as a subgenus of *Euschongastia sensu lato*. A number of genera and subgenera have been added. There are difficulties in correlating these genera with those of Gunther and of Radford. Gunther (1952) himself stated that he was lumping his species into broad genera without subgenera and without making new combinations because of the fluidity of concepts then prevailing. Radford (1954) followed Womersley (1952), but raised many of Womersley's subgenera to genera. Because some of Womersley's sub-

* The names 'harvest-mites' and 'scrub-itch' or 'scrub-typhus' mites are not generally applicable to the larval trombiculids, while the name 'chiggers' is. The risk of confusion with the jigger flea (*Tunga penetrans*) is slight except in Africa, but ambiguity can easily be avoided (Audy, 1954*c*).

genera were based provisionally on post-larval characters and have since been revised, a detailed comparison with Radford's genera is not practicable, but the tabular comparison by Audy (1954*b*: 134) is sufficient for this purpose.

Figs. 1–26 which follow draw attention to some important characters.

LIST OF GENERA AND SUBGENERA, TROMBICULIDAE

Authors are given only for those genera and subgenera which have been raised since Wharton & Fuller (1952) went to press. The numbers in parentheses refer to pages of the present checklist; those without such numbers occur outside the Oriental and Australasian regions. The genera are grouped as far as possible into 'complexes' which are clearly related to each other; a few genera remain ungrouped.

Trombiculinae

Trombicula complex:
- *Anomalaspis* Brennan, 1952
- *Speleocola* Lipovsky, 1952
- *Tecomatlana* (240)
  - subg. *Tecomatlana*
  - subg. *Trisetica* (240)
- *Trombicula* (227)
  - subg. *Crotiscella*
  - subg. *Euschongastoides* Loomis, 1954
  - subg. *Leptotrombidium* s.l. (227)*
  - subg. *Miyatrombicula* Sasa, Kawashima & Egashira, 1952 (233)
  - subg. *Trombicula* s.s. (233)
  - subg. *Trombiculindus* s.l. (234)
  - subg. *Vorcana* Audy, 1956 (236)
- New genus, Vercammen-Grandjean & Brennan (240)

Eutrombicula, *Blankaartia* and *Babiangia* complexes:
- *Eutrombicula* (240)
  - subg. *Eltonella* Audy, 1956 (240)
  - subg. *Eutrombicula* s.s. (241)
- *Fonsecia* (244)
- *Ipotrombicula* Womersley, 1952 (244)
- *Sauriscus*
- *Vercammenia* Audy & Nadchatram, 1957 (244)
- *Blankaartia* [*Tragardhula*] (245)
- *Heaslipia* (245)
- *Neotrombicula* (245)
- *Babiangia* Southcott, 1954 (248)
- *Novotrombicula* (248)
- *Sisecа* Audy, 1956 (247)

Ungrouped genera:
- *Myotrombicula* (248)

Ungrouped genera (*cont.*):
- *Riedlinia* (249)
- *Speotrombicula*

Schongastia complex:
- *Endotrombicula*
- *Oenoschongastia* (251)
- *Phrynacarus*
- *Radfordiana* Womersley, 1952 (251)
- *Schongastia* (249)

Euschongastia and *Ascoschongastia* complexes:
- *Ascoschongastia* (260)
  - subg. *Ascoschongastia* (260)
  - subg. *Laurentella* Audy 1956 (261)
- *Elianella* Vercammen-Grandjean, 1956
- *Euschongastia* (251)
  - subg. *Derrickiella* Audy & Domrow, 1957 (251)
  - subg. *Euschongastia* s.s.
  - subg. *Trombewingia* Fonseca, 1955 (254)
  - subg. *Walchiella* Fuller, 1952 (255)
- *Pseudoschongastia* (264)
- *Helenicula* Audy, 1954 (265)

Neoschongastia complex:
- *Cheladonta* Lipovsky, 1955
- *Mackiena* (268)
- *Neoschongastia* (266)

Ungrouped genera:
- *Doloisia*
- *Guntherana* (270)
- *Schoutedenichia* Jadin & Vercammen-Grandjean, 1954 (270)
- *Traubacarus* Audy & Nadchatram, 1957 (269)

* Including *Mehracula* Sinha, 1954, a synonym, and part of *Plumosicola* Sinha, 1954, which latter also includes species of *Trombiculindus* and is not accepted as a valid subgenus here.

GAHRLIEPIINAE (= WALCHIINAE)*

Gahrliepia complex:
Audya Vercammen-Grandjean, 1956
Gahrliepia (271)
subg. *Gahrliepia* (271)
subg. *Giroudia* Vercammen-Grandjean, 1952

Gahrliepia complex (*cont.*):
subg. *Jadiniella* Vercammen-Grandjean, 1957
subg. *Schongastiella* (275)
subg. *Walchia* (278)

APOLONIINAE

Apolonia
Sauracarella
Womersia

LEEUWENHOEKIINAE

Acomatacarus (281)
subg. *Acomatacarus* (281)
subg. *Austracarus*
subg. *Austrombicula*
subg. *Hyracarus*
subg. *Xenacarus*
Brennanella Radford, 1954†
Chatia
Hannemania (283)
Leeuwenhoekia (281)
subg. *Comatacarus* (281)
subg. *Leeuwenhoekia*
Odontacarus
Parashunsennia Kumada, 1956
Shunsennia Jameson & Toshioka, 1953
Whartonia (283)

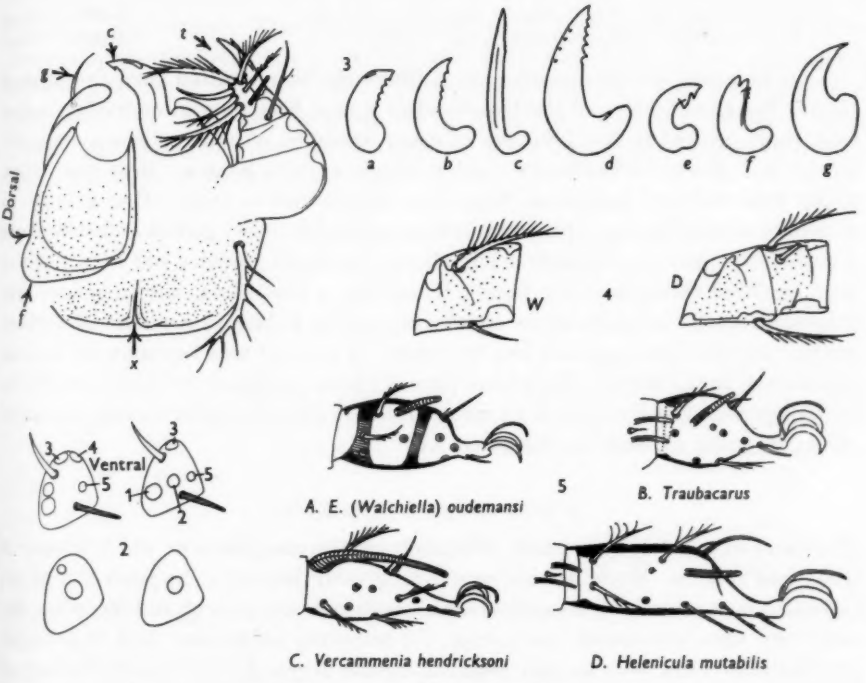
SUBFAMILIES OF TROMBICULIDAE

The family Leeuwenhoekiidae Womersley (1945) and the subfamily Guntheraninae Gunther (1952) were discussed by Audy (1954*b*: 137, 156, 163). The family Leeuwenhoekiidae, which would include the subfamilies Leeuwenhoekiinae and Apoloniinae, was not recognized as a family by Wharton & Fuller and it is not recognized here. The genus *Guntherana* is closely related to *Doloisia* and *Traubacarus*, and possibly through these to *Schoutedenichia* and the Gahrliepiinae. No advantage can be seen in recognizing the Guntheraninae at this stage. The discovery of *Schoutedenichia* has finally broken down the apparent isolation of *Gahrliepia*. This was clearly recognized by Vercammen-Grandjean (1954) who, however, proposed creating a new intermediate subfamily, similar to the Guntheraninae. In the present checklist, the subfamily Gahrliepiinae is retained in a restricted sense beside the *Schoutedenichia* generic complex, and it differs from the Walchiinae of Wharton & Fuller and of Radford (1954) in excluding such genera as *Walchiella*, *Pseudoschongastia* and *Anomalaspis*, which are clearly Trombiculine. Radford included the Walchiinae in the family Leeuwenhoekiidae for the first time, but there are no relationships to recommend this.

* Wharton & Fuller (1952) and also Vercammen-Grandjean (various papers on African species) recognize genera *Gahrliepia*, *Gateria*, *Walchia*, etc., in the subfamily Walchiinae. The present paper, however, being concerned only with the trombiculids from the Oriental and Australasian regions, follows Womersley (1952) and Traub and his colleagues (1954, 1955, 1957), who have described many species from these regions and who consider *Walchia* and the others to be congeneric with *Gahrliepia sensu lato*, as shown above. A universally acceptable arrangement has still to be made.

† The status of this genus requires further investigation. It generally resembles the batchiggers of the genus *Whartonia*, but has 7.7.7 leg-segmentation, according to the description: this would make it an exception in the Leeuwenhoekiinae, which are characterized by 6.6.6 leg-segmentation.

The subfamilies may be keyed out as follows, the number of segments to the legs, which is decided by the separation or fusion of telofemur from basifemur, being shown respectively for legs I, II, III in the form 6.6.6, 7.6.6, or 7.7.7.



Figs. 1–5. Mouthparts and legs.

Fig. 1. Gnathosome showing important diagnostic features. *c*, cheliceral blade (with well-developed tricuspid cap and dorsal toothlet), see Fig. 3; *g*, galeal seta (here nude); *f*, flange to cheliceral base, present in *Walchiella* and occasionally in other chiggers; *t*, palpal tibia (with claw and setae), and tarsus (in this case with tarsala which is always present, subterminala which is sometimes present, and four ventral barbed setae—see Fig. 2); *x*, imperfect fusion of palpal coxae (gnathobase), characteristic of *Walchiella*.

Fig. 2. Palpal tarsi (diagrammatic; ventral and dorsal views) of two similar subgenera of *Euschongastia*. An important generic character.

Fig. 3. Examples of modified cheliceral blades: *a*, *Euschongastia* (*Walchiella*) *oudemansi*; *b*, *Schongastia vieta*; *c*, *Radfordiana rostrata*; *d*, *Acomatacarus* (*Acomatacarus*) sp.nr. *audyi*; *e*, *Traubacarus vercammeni*; *f*, *Oenoschongastia cana*; *g*, *Trombicula* (*Vorcana*) *vorca*.

Fig. 4. Femora of leg III. *W*, fused (e.g. in legs II and III of *Walchiella* and all Gahrliepiines). *D*, not fused (as in most chiggers).

Fig. 5. Tarsus of leg I. *A*, two 'bars' (annular, or in the case of 'semi-bars', semi-annular, thickenings), the distal bar being well developed in *Gahrliepiines*, *Walchiella*, *Derrickiella*, *Schoutedenichia*, and most *Laurentella*; also, posterior proximal placing of microtarsala (most developed in *Walchiella*); *B*, one poorly developed proximal bar, and microtarsala distal to tarsala; *C*, unusually developed tarsala; *D*, distal placing of tarsala, present in all *Helenicula* where it is a generic character.

1. All legs segmented 6.6.6 and 2 setae on coxa I. LEEUWENHOEKIINAE
   Legs segmented 7.7.7 or 7.6.6 (leg I with 7 segments) and 1 seta on coxa I. 2
2. Scutum with paired anterior submedian setae, or with anterior median projection, or with both. APOLONIINAE
   Scutum with one or without median seta, without projection. 3
3. Anteromedian scutal seta absent, legs always 7.6.6. GAHRLIEPIINAE
   Anteromedian scutal seta present, legs usually 7.7.7. TROMBICULINAE

Keys to genera and subgenera have deliberately been omitted from the present paper. The classification of the trombiculids is now becoming more phylogenetic. One result, related to the discovery of many examples of convergence among the larvae, is a greater difficulty in making simple keys to genera. Most characters which were hitherto familiar in keys have been found to recur, often fitfully, in a number of unrelated groups, and it is thus preferable to key genera and subgenera together. Several new characters have been discovered recently but have not yet been explored throughout the family. Therefore, a provisional key to genera and subgenera has been drawn up for circulation, but its publication will await further studies and the testing of the key by usage. A copy of this key may be had on application to the writer. Keys have recently been published by Audy (1956*b*) to various genera, and by Audy & Domrow (1957) to subgenera and species-groups of *Ascoschongastia* s.l. and *Euschongastia* s.l.

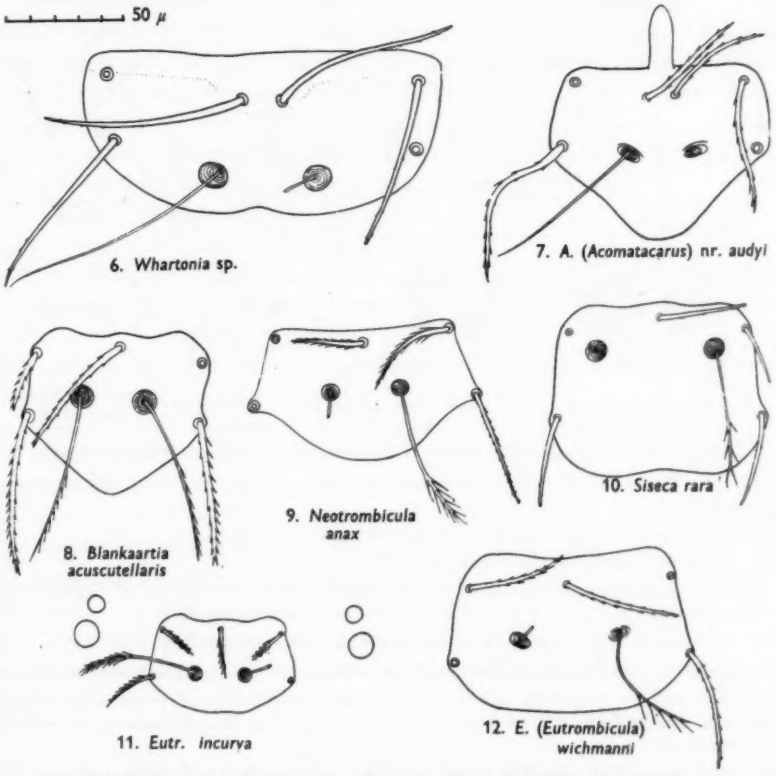
NOTES ON THE CHECKLIST

The value of a checklist depends ultimately on the completeness and accuracy of published records. Such records would be greatly improved by such things as: (i) basing host records on standard checklists, which are quoted, and recording the authority who determined the species; (ii) recording infestation data in a simple standardized form such as that required by the Royal Entomological Society of London for its publications; (iii) recording hosts examined but not found infested as well as those found infested; (iv) recording, whenever possible, an opinion as to the casual or exceptional nature of an infestation (the importance of indicating casual infestations as well as stragglers has been stressed recently by Audy, 1956*b*); (v) revising the host distribution whenever a species is synonymized, for the hosts and locality data of that species may later often remain undetected (e.g. *Guntherana kallipygos* on p. 271); (vi) making particularly clear statements about unattached chiggers, especially those found on man, for even non-parasitic arthropods will swarm temporarily, for example, on to boots, and there is a serious risk that these may have 'man' recorded as hosts, e.g. Radford (1954: 267) records in his list *Homo sapiens* as the sole host of *Guntherana parana* (= *kallipygos*), but this refers to larvae collected from boots.

Host names. Authorities for the host names used in this checklist are: Mammals—Ellerman & Morrison-Scott (1951), Chasen (1940) as amended by Ellerman & Morrison-Scott (1955), Iredale & Troughton (1934) (whose nomenclature is followed by Troughton, 1951), Laurie & Hill (1954). Reptiles—Smith (1935), Tweedie (1953). Birds—Gibson-Hill (1949, and personal communications). I am grateful to my colleague Mr J. L. Harrison for his help in checking host

names. Hosts are grouped broadly as Carnivores, Insectivores, Marsupials, Primates, Rodents, Ungulates, Reptiles, Birds and Arthropods. A valuable summary of relevant data on host ecology and behaviour is given by Harrison (1957*b*).

It is necessary to record some of the more important revisions of host names, especially since these revisions have reduced the number of species and greatly clarified relationships. The following host names are those encountered fairly frequently in the literature, listed alphabetically with their correct equivalents according to the authorities quoted. Wharton & Fuller list a number of others in their Host List. Most records published by American workers are based on Allen (1938, 1940), some of whose names are listed below with their equivalents according to Ellerman & Morrison-Scott.

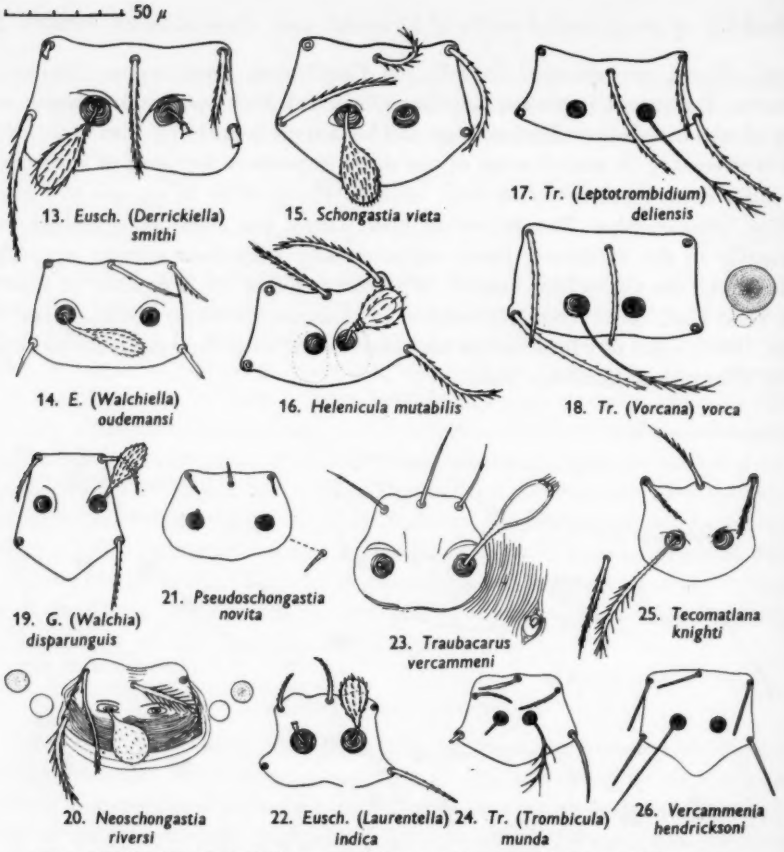


Figs. 6–12. Characteristic scuta. Eyes are shown only for special reasons.

Figs. 6, 7. Leeuwenhoekiines with paired anteromedian setae; one scutum showing anterior median process. All Trombiculines have one anteromedian seta, all Gahrliepiines (Fig. 19) have none.

Figs. 8, 9. Two related genera, scuta chosen to exaggerate the differences. Note that Fig. 8 has scutum extended anterior to line joining antero-lateral setae, giving rise to 'shoulders' which are also shown in Figs. 10–12, 20, 22 and 24.

Figs. 10–12. Three related groups of reptile-chiggers, all with shoulders. The posterior eye in Fig. 11 is larger than the anterior, which is unusual.



Figs. 13–26. Characteristic scuta. Eyes are shown only for special reasons.

Figs. 13, 14. Two related subgenera: scuta of similar shape but with characteristically different setae.

Fig. 15. A genus of reptile-chiggers with subpentagonal scuta.

Fig. 16. Sensillary bases approximated, a generic character.

Figs. 17, 18. Barbed sensillae and subrectangular scuta characteristic of *Leptotrombidium* and related groups; the large eye in Fig. 18 is a subgeneric character.

Fig. 19. Absence of anteromedian seta characterizes the Gahrliepiines.

Fig. 20. A genus of bird-chiggers with scuta apparently part-submerged beneath cuticular striae.

Fig. 21. Example of reduction of scutum resulting in isolation of postero-lateral setae—characteristic also of *Traubacarus* (Fig. 23), *Tecomatlana* (Fig. 25), *Ascoschongastia*, *Sauriscus* and *Anomalaspis*.

Fig. 22. Note 'shoulders' (see Fig. 8) and sensillae, and compare with related species, Fig. 24.

Fig. 23. Reduction of scutum (see Fig. 21) is in this case associated with endoparasitism (intranasal); the isolated postero-lateral seta, as with dorsal setae (not shown), is on a small plate, which is a specific character.

Fig. 24. Contrast with Figs. 17, 18, which species are at present accommodated in the genus *Trombicula* but are probably generically distinct from Fig. 24—the group to which *munda* belongs is more closely related to *Laurentella* (Fig. 22) and possibly *Tecomatlana* (Fig. 25) than it is to *Leptotrombidium* (Fig. 17) or *Vorcana* (Fig. 18).

Fig. 25. A genus of bat-chiggers—note the thickened but not swollen sensilla.

Fig. 26. This is an endoparasitic (hypodermal) species and the scutum is poorly chitinized while the setae and sensillae are nude.

Rodents

Gunomys bengalensis is *Bandicota bengalensis* (Gray & Hardwicke).

Leggada booduga fulvidiventris (from Ceylon) is *Mus cervicolor fulvidiventris* (Blyth).

Melomys stalkeri is *M. rufescens stalkeri* (Thomas).

Millardia millardia is *M. meltada* (Gray).

Mogera wogura is *Talpa micrura* Hodgson.

Mus has sometimes been used unjustifiably as an equivalent of 'unidentified mouse', or 'small rat'—to the writer's knowledge, an author's 'Mouse' referring in one case to *Alticola roylei*, and in another to a young unidentified rat, has in both cases been translated wrongly into '*Mus* sp.'

Mus bactrianus kakhyensis is *M. musculus homourus* (Hodgson).

Rattus has sometimes been used unjustifiably as an equivalent of 'unidentified rat'. There are, for example, over thirty genera of rodents in the Palaearctic and Oriental regions which might be described as 'rat' or 'mouse'.

Rattus browni is *R. exulans browni* (Alston).

Rattus flavipectus, subspp. *flavipectus, yunnanensis*, are *R. rattus* subspp.

Rattus malaisia is *R. canus malaisia* Kloss.

Rattus mordax is *R. ruber mordax* (Thomas).

Rattus murrayi is *R. greyi murrayi* Thomas.

Rattus norvegicus norvegicus as used by Radford (1946*a*) for the rat on Addu Atoll, Maldive Is., was a field identification probably of *R. r. alexandrinus*.

Rattus pellax is *R. rajah pellax* Miller.

Rattus rattus argentiventer is *R. argentiventer* (Robinson & Kloss) (after J. L. Harrison, to be published).

Rattus rattus nr. *brunneusculus* (from Manipur) is *R. r. bullocki* Roonwal.

Rattus rattus jalorensis is *R. jalorensis* (Bonhote) (after J. L. Harrison to be published).

Rattus rattus rufescens as used by Radford (1946*b*) for hosts from Manipur was a provisional field identification and should be *R. r. bullocki* Roonwal.

Rattus ringens is *R. ruber ringens* (Peters & Doria).

Rattus surifer is *R. rajah surifer* Miller.

Rattus youngi is *R. culmorum youngi* Thomas.

Uromys lamington (or *lamingtonensis*) is *U. caudimaculatus aruensis* Gray.

Insectivores

Anourosorex squamipes assamensis is *A. s. squamipes* Milne-Edwards.

Crocidura, *Sorex*, and *Suncus* have wrongly been regarded by some authors as interchangeable.

Crocidura vorax is *C. russula vorax* Allen.

Suncus coeruleus is *S. murinus* (L.).

Tupaia belangeri is *T. glis* Diard.

Ungulates—Artiodactyla

Tragulus kanchil (Lesser Mouse-deer) is *T. javanicus* Osbeck; *T. javanicus* auct. nec Osbeck (Larger Mouse-deer, absent from Java) is *T. napu* Cuvier.

Marsupials

Echymipera cockerelli and *E. doreyana* are both *E. kalubu kalubu* (Lesson).
Isoodon Desmarest is a synonym of *Thylacis* Illiger.
Phascogale includes only two unusual species; most marsupial mice referred to *Phascogale* are species of *Antechinus*.

Host-list. Wharton & Fuller (1952: 116–38) give a classified list of hosts with chiggers recorded on them. This is based on a checklist which, like the present one, suffers from a serious defect in that the range of hosts recorded for any species of chigger is always very incomplete and usually strongly biased (see Audy, 1954*a*: 33). For example, *Rattus bowersi* was not recorded as a host of chiggers before Wharton & Fuller's lists went to press, but we could now list this rat with the following published records of chiggers: *Trombicula consueta*, *T. deliensis*, *T. muridia*, *Ascoschongastia indica*, *A. sellnicki*, *Traubacarus browningi*, *T. intermedia*, (?)*T. manipurensis*, *T. varmai*, *T. vercammeni*, *Gahrliepia birella*, *G. brennani*, *G. fletcheri*, *G. insigne*, *G. ventralis*. This rat, however, is in fact infested with the greatest variety of chiggers yet encountered by us on a single species of host, the present record being over thirty species of chiggers on *R. bowersi*, and on one occasion thirteen species on a single individual. It is obviously too early to publish here an additional host-list, which would be seriously lacking in balance. It is proposed instead to publish at a later date a critical host-list relating to a single country (Malaya) and to determine its implications. Other local host-lists may then be drawn up, guided by these implications.

Place names. Countries are as far as possible grouped zoogeographically. The smaller islands in the Pacific Ocean are grouped under the heading OCEANIA. Records for BURMA are grouped into those from the north and the south, because these two parts differ considerably and have been quite separately sampled. Records for BORNEO refer wholly to British North Borneo and Sarawak. Records for INDIA are grouped as follows: (i) INDIA, North—from the Kumaon Himalayas to Kashmir and Jammu (collections by Col. Sadari Lal Kalra in 1946–7); (ii) INDIA, North-east—from the Manipur (Imphal) area and Northern Assam, all near the Indo-Burma border; (iii) INDIA, Peninsular—from the rest of India. Approximately a hundred separate collecting efforts of reasonable size have been made in the regions covered by this paper. Information about the localities and sizes of these collections has been tabulated and discussed by Audy (1954*a*).

Authors. In the following checklist references given in the checklist of Wharton & Fuller (1952) are not repeated. Abbreviations used for authors and co-authors are as follows: A., Audy; Dom., Domrow; E., Evans; F., Fuller; Jad., Jadin; Jam., Jameson; Har., Harrison; Gun., Gunther; M., Morrow; Nad., Nadchatram; Rad., Radford; Suy., Suyemoto; Tosh., Toshioka; Verc., Vercammen-Grandjean; W., Wharton; Wom., Womersley.

System of references. Where further information about a paper is considered necessary it is given as follows, being separated from the year of publication by a colon: (i) page references, (ii) in parentheses, plate or page reference to figures when these are separated from the text (Womersley, 1952, omits references to figures in his index but these are given by Womersley & Audy, 1957), and (iii) a letter or letters to indicate the kind of information the paper contains. These indicator letters are those of Wharton & Fuller 1952: 40 with some additions, and they are as follows: a—anatomy; b—biology; c—chorology or distribution (on hosts and/or in space); D—original description; d—redescription or partial description; k—in a key (but only keys in a few papers are indicated thus); l—in a list or checklist (but not shown for the major checklists); m—medical; s—species synonymized; t—taxonomy; v—veterinary; x—a corrigendum or addendum.

Date of checklist. An effort has been made to make this checklist refer generally to all publications up to the end of 1955, with the addition of all papers published or in press in 1956 which have been consulted by the writer at least in manuscript.

CHECKLIST OF TROMBICULIDAE OF THE ORIENTAL AND AUSTRALASIAN REGIONS

This list contains only references and records which are additional to those already recorded from these regions in the checklist of world trombiculids of Wharton & Fuller (1952).

Notes on the arrangement of this checklist are given in the preceding section.

Subfamily Trombiculinae Ewing

Genus **Trombicula** Berlese, 1905

Type: *Trombicula minor* Berlese, 1905

Subgenus **Leptotrombidium** Nagayo, Miyagawa, Mitamura & Imamura, 1916 incl. **Mehracula** Sinha, 1954

Type: *Trombidium akamushi* Brumpt, 1910

Trombicula (Leptotrombidium) akamushi* (Brumpt)

Trombicula (*Leptotrombidium*) *akamushi*, Wom. 1952: 67 (6) dc, 345 (90) dNA, W. & F. 1952: 51, Fuller 1952: 23 (dtcm), Sasa 1953: 412 (434–5) dNA, Sasa & Jam. 1954: 292 (287) dc, Traub & A. 1954*a*: 74 (70, 71) dct, Harrison 1954 (b), 1956*a* (c), 1956*b* (c), 1957 (b), Suy. & Tosh. 1955 (c), Hammen 1956: 37 (d), Audy 1956*b*: 58, 72 (c), 1956*d*: 93 (dc), 1957 (bcm), Wom. & A. 1957 (xl).

Trombicula akamushi, IMR 1949: 63, 1951: 50 (bc), Gun. 1952: 10.

Trombicula fletcheri, Rad. 1954: 260.

Leptotrombidium akamushi, Rad. 1954: 260.

* Although *T.* (*L.*) *obscura* Wom. & Heaslip is regarded as a synonym of *akamushi*, this form may be distinct and is discussed by Wom. & Audy (1957).

Records. BORNEO. Rodents—*Rattus r. diardi.* Insectivores—*Tupaia minor, T. montana.* Bird—Unidentified chick. MALAYA. Birds—*Centropus sinensis eurycercus, Excalfactoria c. chinensis, Poliolimnias c. cinereus,* unidentified Rails, *Rallus striatus gularis, Turnix suscitator atrogularis.* Reptile—Single straggler on *Mabuya multifasciata.* NEW GUINEA. Rodent—*Rattus ruber mordax.*

Trombicula (Leptotrombidium) baluensis Traub & Audy

Trombicula (*Leptotrombidium*) *baluensis* Traub & A., 1954*a*: 48 (58, 59) D, Audy 1954*b*: 141 (t).

Records. BORNEO. Rodent—*Dremomys everetti.* Insectivores—*Crocidura* sp., *Hylomys suillus.*

Trombicula (Leptotrombidium) bhimtalensis Womersley

Trombicula (*Leptotrombidium*) *bhimtalensis* Wom., 1952: 60 (7) D, Wom. & A. 1957 (xl).

Leptotrombidium bhimtalensis, Rad. 1954: 260.

Records. INDIA, North. Insectivore—Shrew.

Trombicula (Leptotrombidium) bodensis Gunther

Trombicula (*Leptotrombidium*) *bodensis*, Wom. 1952: 111 (18) d, Wom. & A. 1957 (xl).

Trombicula (*Trombicula*) *bodensis*, W. & F. 1952: 62.

Trombicula bodensis, Gun. 1952: 18, Rad. 1954: 250.

Records. BORNEO. Rodent—*Callosciurus hippurus.*

Trombicula (Leptotrombidium) burmensis Ewing

Trombicula (*Leptotrombidium*) *burmensis*, Wom. 1952: 74 (9) d, 348 (91) DN, Wom. & A. 1957 (xl).

Trombicula (*Trombicula*) *burmensis*, W. & F. 1952: 63.

Trombicula burmensis, Gun. 1952: 16.

Leptotrombidium burmensis, Rad. 1954: 260.

Records. INDIA, North-east. Rodent—*Rattus r. bullocki.*

Trombicula (Leptotrombidium) deliensis (Walch)

Trombicula (*Leptotrombidium*) *deliensis*, Wom. 1952: 62 (6) dc, 342 (90) dNA, IMR 1949 to 1954 incl. (bc), Audy 1952: 133 (c), 1954*a*: 34 (bcm), 1954*b*: 142 (bc), 1954*c*, 1956*b*: 58, 72 (c), 1956*d*: 93 (dc), 1957 (bcm), W. & F. 1952: 52, Audy *et al.* 1953 (bcm), Audy & Har. 1954: 17 (c), Traub & A. 1954*a*: 74 (68, 69) dtc, Traub *et al.* 1954 (cn), Harrison 1954 (b), 1956*a*, *b*, *c* (bc), 1957 (b), Tanaka 1955 (t), Hammen 1956 (dm), Wom. & A. 1957 (xl).

Trombicula akamushi var. *deliensis*, Fuller 1925: 36 (dtcm).

Trombicula buloloensis, Rad. 1954: 250.

Trombicula walchi, Rad. 1954: 258.

Leptotrombidium deliensis, Rad. 1954: 260.

Records. BURMA, South. Rodents—*Bandicota bengalensis, Callosciurus p. pygerythrus, Rattus r. khyensis.* Insectivore—*Suncus murinus.* INDIA, North-east. Rodents—*Callosciurus erythraeus erythrogaster, C. pygerythrus lokroides, Dremomys lokriah, Hadromys humei, Mus* spp., *Rattus bowersi, R. manipulus, R. niviventer mentosus, R. r. bullocki.* Insectivores—*Anourosorex squamipes, Suncus murinus, Tupaia glis.* Carnivores—Domestic cat, *Herpestes ?urva, Mustela* sp. Ungulate—*Muntaiacus muntjak.* Primate—*Macaca assamensis.* Bird—Laughing thrush (Timaliid). INDIAN OCEAN, Car Nicobar. Rodent—*R. rattus* subsp. BORNEO. Rodents—*Callosciurus hippurus, C. lowii, Dremomys everetti, Nannosciurus whiteheadi, Rattus exulans, R. mulleri, R. r. diardi.* Insectivores—*Dendrogale melanura baluensis, Tupaia minor, T. montana.* Arthropod—stragglers on a tipulid fly. MALAYA. Rodents—*Lariscus insignis, Rattus r. jarak.* Bats—*Cheiromeles torquatus, Taphozous melanopogon.* Birds—*Poliolimnias c. cinereus,* unidentified Rail, *Turnix suscitator atrogularis.*

Trombicula (Leptotrombidium) dux Womersley

Trombicula (*Leptotrombidium*) *dux* Wom., 1952: 57 (5) D, Wom. & A. 1957 (xl).
Leptotrombidium dux, Rad. 1954: 260.
Records. INDIA, North. Rodents—*Rattus niviventer*; 'brown rat'.

Trombicula (Leptotrombidium) fulleri Ewing

Trombicula (*Leptotrombidium*) *fulleri*, Wom. 1952: 69 (8) dx, 347 (92) DN, Wom. & A. 1957 (xl).
Trombicula (*Leptotrombidium*) *deliensis*, in part, W. & F. 1952: 53 (ls).
Trombicula fulleri, Gun. 1952: 16.
Leptotrombidium fulleri, Rad. 1954: 260.
Records. INDIA, North-east. Rodents—*Rattus r. bullocki.*

Trombicula (Leptotrombidium) gliricolens (Hirst)

Trombicula gliricolens, Wom. 1952: 134 (25) d, Gun. 1952: 19.
Trombicula (*Trombicula*) *gliricolens*, W. & F. 1952: 65.
Eutrombicula (*sic!*) *gliricolens*, Rad. 1954: 261.
Trombicula (*Leptotrombidium*) *gliricolens*, Audy 1954*b*: 141 (tx), Wom. & A. 1957 (xl).
Records. INDIA, North. Rodents—*Rattus rattoides, Rattus* sp.

Trombicula (Leptotrombidium) jayewickremei Womersley

Trombicula (*Neotrombicula*) *jayewickremei* Wom., 1952: 138 (26) D, 356 (94) DN.
Trombicula (*Leptotrombidium*) *jayewickremei*, Audy 1954*b*: 142 (tx), Wom. & A. 1957 (xl).
Neotrombicula jayewickremei, Rad. 1954: 259.
Records. CEYLON. Rodents—*Rattus r. kandiyanus.*

Trombicula (Leptotrombidium) kalrai Radford, new status

Trombicula kalrai Rad., 1953*a*: 231 (d), 1954: 257, Audy 1954*b*: 148.
Records. INDIA, North. Rodent—'Rat (*Rattus* sp.)'.

Trombicula (Leptotrombidium) keukenschrijveri (Walch)

Trombicula (? *Leptotrombidium*) *keukenschrijveri*, Wom. 1952: 49 (3) d.

Trombicula (*Leptotrombidium*) *keukenschrijveri*, W. & F. 1952: 53, Harrison 1954: 180 (b), 1957 (b), Audy 1954*b*: 142 (t), 1956*d*: 93 (cd), Dom. 1957*a*: ... DN, Wom. & A. 1957 (xl).

Trombicula keukenschrijveri, Gun. 1952: 17.

Leptotrombidium keukenschrijveri, Rad. 1954: 260.

Records. MALAYA. Rodents—*Rattus bowersi*, *R. jalorensis*, *R. mulleri*, *R. sabanus*. Insectivore—*Crocidura malayana*. Carnivore—*Prionodon linsang*.

Trombicula (Leptotrombidium) lanceolata Womersley

Trombicula (*Leptotrombidium*) *lanceolata* Wom., 1952: 54 (4) D, Audy 1954*b*: 142 (t), Wom. & A. 1957 (xl).

Leptotrombidium lanceolata, Rad. 1954: 260.

Records. BURMA, South. Rodents—*Bandicota bengalensis*, *Rattus r. khyensis*.

Trombicula (Leptotrombidium) langati Audy & Womersley

Trombicula (*Leptotrombidium*) *langati* Audy & Wom. 1957: ... D.

Records. MALAYA. Carnivore—*Paradoxurus hermaphroditus*. Insectivore—*Tupaia glis*, *T. minor*. Rodents—*Rattus annandalei*, *R. bowersi*, *R. jalorensis*, *R. mulleri* (chief host), *R. sabanus*; *Callosciurus tenuis*, *Rhinosciurus laticaudatus*.

Trombicula (Leptotrombidium) longiseta Womersley

Trombicula (*Leptotrombidium*) *longiseta* Wom., 1952: 61 (8) D, 348 (92) DN, Audy 1954*b*: 142 (t), Wom. & A. 1957 (xl).

Leptotrombidium longiseta, Rad. 1954: 260.

Records. INDIA, North-east. Rodent—*Rattus r. bullocki*. BURMA, North. Rodent—Unidentified.

Trombicula (Leptotrombidium) macacus Womersley

Trombicula (*Leptotrombidium*) *macacus* Wom., 1952: 56 (5) D, Audy 1954*b*: 142 (t), Wom. & A. 1957 (dxl).

Leptotrombidium macacus, Rad. 1954: 260.

Records. INDIA, North-east. Primate—*Macaca assamensis*.

Trombicula (Leptotrombidium) micula Traub & Audy

Trombicula (*Leptotrombidium*) *micula*, Traub & A., 1954*a*: 51 (72, 73) D, Audy 1954*b*: 142 (t).

Records. BORNEO. Rodents—*Callosciurus notatus*, *Nannosciurus melanotis*.

Trombicula (Leptotrombidium) muridia Womersley

Trombicula muridia Wom., 1952: 121 (20) D, Audy 1952: 133 (c), Rad. 1954: 257.

Trombicula (*Leptotrombidium*) *muridia*, Audy 1954*b*: 142 (t), 1956*d*: 93 (c), Harrison 1957 (b), Wom. & A. 1957 (xl).

Records. MALAYA. Rodents—*Lariscus insignis*, *Rattus bowersi*, *R. mulleri*, *R. sabanus*, *Rhinosciurus laticaudatus*. Bat—*Cheiromeles torquatus*.

Trombicula (Leptotrombidium) myzantha Womersley

Trombicula (*Leptotrombidium*) *myzantha* Wom., 1952: 71 (9) D, 347 (91) DN, Audy 1954*b*: 142 (t), Wom. & A., 1957 (xl).

Leptotrombidium myzantha, Rad. 1954: 260.

Records. AUSTRALIA. Birds—*Cracticus torquatus* (?), *Cuculus pallidus*. NEW GUINEA (?). Bird—Fruit pigeon. OCEANIA, Solomons. Bird—Pigeon.

Note. The forms *owiensis* and *bougainvillensis*, from two islands, proposed by Womersley, might be distinct species or subspecies.

Trombicula (Leptotrombidium) parapalpalis Womersley

Trombicula (*Leptotrombidium*) *parapalpalis* Wom., 1952: 55 (10) D, Audy 1954*b*: 142 (t), Wom. & A. 1957 (xl).

Leptotrombidium parapalpalis, Rad. 1952: 260.

Records. INDIA, North. Rodent—Unidentified rat.

Trombicula (Leptotrombidium) pelta Womersley

Trombicula pelta Wom., 1952: 137 (28) D, Rad. 1954: 258.

Trombicula (*Leptotrombidium*) *pelta*, Audy 1954*b*: 142 (t), Wom. & A. 1957 (xl).

Records. INDIA, North. Rodent—Unidentified rat.

Trombicula (Leptotrombidium) pilalta Traub & Audy

Trombicula (*Leptotrombidium*) *pilalta* Traub & A., 1954*a*: 50 (64, 65) D, Audy 1954*b*: 142 (t).

Records. BORNEO. Rodent—*Rattus mulleri*.

Trombicula (Leptotrombidium) pipellae Traub & Audy

Trombicula (*Leptotrombidium*) *pipellae* Traub. & A., 1954*a*: 49 (62, 63) D, Audy 1954*b*: 142 (t), Dom. 1957*a*: ... DN.

Records. BORNEO. Rodents—*Callosciurus notatus*, *Rattus alticola*, *R. mulleri*. Insectivore—*Echinosorex gymnurus*. Carnivores—*Helictis everetti*, *Prionodon linsang gracilis*.

Trombicula (Leptotrombidium) puta Womersley

Trombicula (*Leptotrombidium*) *puta* Wom., 1952: 57 (10) D, Audy 1954*b*: 142 (t), Wom. & A. 1957 (xl).

Leptotrombidium puta, Rad. 1954: 260.

Records. INDIA, North. Rodents—*Apodemus flavicollis ?wardi*, *Rattus ?rattus* subsp.

Trombicula (Leptotrombidium) radfordi (Sinha)

Mehracula radfordi Sinha, 1954: 330 (D).

Trombicula (*Leptotrombidium*) *radfordi*, Sinha 1957: 295 (dx).

Records. INDIA, North-east. Rodent—*Dremomys macmillani*. Carnivore—*Herpestes urva*.

Trombicula (Leptotrombidium) robusta Gunther

Trombicula (*Leptotrombidium*) *robusta*, Wom. 1952: 73 (9) d, Audy 1954*b*: 142 (t), Wom. & A. 1957 (xl).

Trombicula robusta, Gun. 1952: 20.

Trombicula (*Trombicula*) *robusta*, W. & F. 1952: 69.

Leptotrombidium robusta, Rad. 1954: 260.

Records. NEW GUINEA. Bird—'Thrush-like bird'.

Trombicula (Leptotrombidium) roonwali (Sinha)

Mehracula roonwali Sinha, 1954: 330 (D).

Trombicula (*Leptotrombidium*) *roonwali*, Sinha 1957: 295 (dx).

Records. INDIA, North-east. Insectivore—*Tupaia glis belangeri*.

Trombicula (Leptotrombidium) sylvestris Audy & Traub

Trombicula sylvestris Audy & T., 1952: 325 (D), Wom. 1952: 133 (24) d, Rad. 1954: 258.

Trombicula (*Leptotrombidium*) *sylvestris*, W. & F. 1952: 55, Audy 1954*b*: 142 (t), 1956*d*: 93 (c), Wom. & A. 1957 (xl).

Trombicula (Leptotrombidium) tarsala Traub & Audy

Trombicula (*Leptotrombidium*) *tarsala* Traub & A., 1954*a*: 51 (66, 67) D, Audy 1954*b*: 142 (t).

Records. BORNEO. Rodent—*Rattus mulleri*.

Trombicula (Leptotrombidium) tithwalensis Womersley

Trombicula tithwalensis Wom., 1952: 123 (21) D, Rad. 1954: 258.

Trombicula (*Leptotrombidium*) *tithwalensis*, Audy 1954*b*: 142 (t), Wom. & A. 1957 (xl).

Records. INDIA, North. Rodents—Unidentified 'rat' and 'mouse'.

Trombicula (Leptotrombidium) villosa Womersley

Trombicula (*Leptotrombidium*) *villosa* Wom., 1952: 60 (7) D, Audy 1954*b*: 142 (t), Wom. & A. 1957 (xl).

Leptotrombidium villosa, Rad. 1954: 260.

Records. INDIA, North. Rodent—'Rat'.

Subgenus **Miyatrombicula** Sasa, Kawashima & Egashira, 1952

Type: *Trombicula kochiensis* Sasa *et al.*, 1952

Trombicula (Miyatrombicula) buxtoni Womersley

Trombicula buxtoni Wom., 1952: 124 (22) D, Audy 1954*b*: 147 (t), Rad. 1954: 250.

Trombicula (*Miyatrombicula*) *buxtoni*, Wom. & A. 1957 (xl).

Records. INDIA, North. Rodent—'Yellow coloured rat'.

Trombicula (Miyatrombicula) rajoriensis Womersley

Trombicula rajoriensis Wom., 1952: 129 (20) D, Rad. 1954: 258.

Trombicula (*Trombicula*) *rajoriensis*, Audy 1954*b*: 147 (t), Wom. & A. 1957 (xl).

Trombicula (*Miyatrombicula*) *rajoriensis*, Wom. & A. 1957 (xl).

Records. INDIA, North. Rodent—'Rat'.

Subgenus **Trombicula** Berlese, 1905

Type: *Trombicula minor* Berlese, 1905

Trombicula (Trombicula) batui Philip & Traub

Trombicula batui, Wom. 1952: 105 (17) d, Audy 1952: 134 (dc), 146 (k), Rad. 1954: 250.

Trombicula (*Trombicula*) *batui*, W. & F. 1952: 62, Audy 1954*b*: 147 (tx), 1956*d*: 92 (c), Wom. & A. 1957 (xl).

Records. MALAYA. Bat—*Taphozous melanopogon.*

Trombicula (Trombicula) insolli Philip & Traub

Trombicula insolli, Wom. 1952: 128 (23) d, Audy 1952: 134 (dc), 146 (k), 1954*b*: 147 (t), 1956*d*: 92 (cd), Gun. 1952: 18, Loomis 1954: 924, Rad. 1954: 257,

Trombicula (*Trombicula*) *insolli*, W. & F. 1952: 66, Wom. & A. 1957 (xl).

Records. MALAYA. Bats—*Taphozous melanopogon.*

Trombicula (Trombicula) khurdangensis Womersley

Trombicula khurdangensis Wom., 1952: 119 (20) D.

Trombicula kurdangensis, Rad. 1954: 257.

Trombicula (*Trombicula*) *khurdangensis*, Wom. & A. 1957 (xl).

Records. INDIA, North. Rodent—'Rat'.

Trombicula (Trombicula) minor Berlese

Trombicula (*Trombicula*) *minor*, Wom. 1952: 328 (87) dA, Gun. 1951 (tcx), 1952: 5, W. & F. 1952: 61, Audy 1954*b*: 138 (t), Wom. & A. 1957 (xl).

Trombicula minor, Rad. 1954: 257.

Trombicula (Trombicula) munda Gater

Trombicula (*?Neotrombicula*) *munda*, Wom. 1952: 106 (17) d.

Trombicula (*Trombicula*) *munda*, W. & F. 1952: 67, Audy 1954*b*: 143, 147 (t), 1956*d*: ... (cd), Domrow 1951*a*, *b* (t), Wom. & A. 1957 (xl).

Trombicula munda, Gun. 1952: 18, Fuller 1952: 88 (d), Rad. 1954: 257.

Neotrombicula munda, Rad. 1954: 259.

Records. CEYLON. Rodent—*Rattus r. kandiyanus*.

Trombicula (Trombicula) schmitzi (Oudemans)

Trombicula schmitzi, Wom. 1952: 425 (d), Fuller 1952: 95 (dt), Rad. 1954: 258.

Trombicula (*Trombicula*) *schmitzi*, W. & F. 1952: 70, Audy 1954*b*: 147 (t), Wom. & A. 1957 (xl).

Trombicula (Trombicula) spicea Gater

Trombicula (*Neotrombicula*) *spicea*, Wom. 1952: 107 (17) d, 360 (96) DN.

Trombicula (*Trombicula*) *spicea*, W. & F. 1952: 70, Audy 1954: 143, 147 (t), 1956*d*: 92 (cd), Wom. & A. 1957 (xl).

Trombicula spicea, Gun. 1952: 18.

Neotrombicula spicea, Rad. 1954: 259.

Records. CEYLON. Rodent—*Rattus r. kandiyanus*.

Subgenus **Trombiculindus*** Radford, 1948

incl. **Plumosicola** Sinha, 1954, in part

Type: *Trombiculindus squamosus* Radford, 1948

Trombicula (Trombiculindus) cuneatus (Traub & Evans)

Trombicula cuneata, Wom. 1952: 139 (27) d.

Trombicula (*Trombiculindus*) *cuneatus*, W. & F. 1952: 61, Audy 1954*b*: 143 (t), Wom. & A. 1957 (xl).

Trombiculindus cuneatus, Audy *et al.* 1953: 34 (c), Rad. 1954: 263, Sinha 1954: 334 (t).

Records. INDIA, North-east. Insectivore—*Suncus murinus fulvocinereus*.

Trombicula (Trombiculindus) foliaceus (Traub & Evans)

Trombicula foliacea, Wom. 1952: 142 (28) d.

Trombicula (*Trombiculindus*) *foliaceus*(-*a*), W. & F. 1952: 61, Audy 1954*b*: 143 (t), Wom. & A. 1957 (xl).

Trombiculindus foliaceus, Audy *et al.* 1953: 34 (c), Rad. 1954: 263, Sinha 1954: 334 (t).

* *Trombiculindus* would at the most be a subgenus of *Leptotrombidium* if the latter is restored to generic rank.

Trombicula (Trombiculindus) fordi Womersley

Trombicula (*Neotrombicula*) *fordi* Wom., 1952: 75 (11) D, 361 (96) DN.

Trombicula (*Trombiculindus*) *fordi*, Audy 1954*b*: 143 (t), Audy *et al.* 1953 (c), Wom. & A. 1957 (xl).

Records. INDIA, North-east. Rodents—*Rattus r. bullocki*, 'Black rat with grey belly'. Insectivores—*Suncus murinus*.

Trombicula (Trombiculindus) hastata Gater

Trombicula (*Neotrombicula*) *hastata*, Wom. 1952: 77 (12) d, 355 (94) DN.

Trombicula (*Trombicula*) *hastata*, W. & F. 1952: 66.

Trombicula (*Trombiculindus*) *hastata*, Audy 1954*b*: 143 (t), 1956*d*: 93 (cd), Har. 1957 (b), Wom. & A. 1957 (xl).

Neotrombicula hastata, Rad. 1954: 259.

Euschongastia hastata (*sic*!), Rad. 1954: 265.

Trombiculindus (*Plumosicola*) *hastata*, Sinha 1954: 334.

Records. MALAYA. Rodents—*Rattus sabanus* (*Note*: the type host is *R. rajah surifer*).

Trombicula (Trombiculindus) plumosa Radford

Trombicula plumosa Rad., 1953*a*: 231 (D), 1954: 258.

Trombicula (*Trombiculindus*) *plumosa*, Audy 1954*b*: 143 (t).

Trombiculindus (*Plumosicola*) *plumosa*, Sinha 1954: 334.

Records. INDIA, North-east. Rodent—*Callosciurus p. pygerythrus*.

Trombicula (Trombiculindus) pruthi (Sinha)

Trombiculindus pruthi Sinha, 1954: 332.

Records. INDIA, North-east. Rodent—*Dremomys macmillani*. Carnivore—*Herpestes urva*.

Trombicula (Trombiculindus) squamifera Womersley

Trombicula squamifera Wom., 1952: 140 (27) D.

Trombicula (*Trombiculindus*) *squamifera*, Audy 1954*b*: 143, Wom. & A. 1957 (xl).

Trombiculindus squamifera, Rad. 1954: 263, Sinha 1954: 333 (t).

Records. INDIA, North. Rodent—*Alticola roylei*.

Trombicula (Trombiculindus) squamosus (Radford)

Trombicula squamosa, Wom. 1952: 141 (28) d, Gun. 1952: 19.

Trombicula (*Trombiculindus*) *squamosa*, W. & F. 1952: 61, Audy 1954*b*: 143 (t), Wom. & A. 1957 (xl).

Trombiculindus squamosus, Rad. 1954: 263, Sinha 1954: 334 (t).

Trombicula (Trombiculindus) traubi Womersley

Trombicula (*Neotrombicula*) *traubi* Wom., 1952: 76 (11) D.
Trombicula (*Trombiculindus*) *traubi*, Audy 1954*b*: 144 (t), Wom. & A. 1957 (xl).
Neotrombicula traubi, Rad. 1954: 260.
Records. INDIA, North-east. Primate—*Macaca assamensis.* INDIA, North. Rodent—'Rat'.

Subgenus **Vorcana** Audy, 1956

Type: *Trombicula vorca* Traub & Audy, 1954

Trombicula (Vorcana) corvi Hatori

Trombicula corvi, Wom. 1952: 127 (23) d, Gun. 1952: 14, Traub & A. 1954*b*: 48 (t), Rad. 1954: 250.
Trombicula (*Trombicula*) *corvi*, W. & F. 1952: 64.
Trombicula (*vorca*-group) *corvi*, Audy 1954*b*: 148 (t).
Trombicula (*Vorcana*) *corvi*, Audy 1956*b*: 52 (tx), Wom. & A. 1957 (dxl).

Note. Audy (1956*b*) considers that the original description of the sensillae as being barbed on the proximal instead of the distal half is a *lapsus calamae.*

Trombicula (Vorcana) densipiliata Walch

(1) **Trombicula (Vorcana) densipiliata densipiliata** Walch
Trombicula densipiliata, Wom. 1952: 126 (t), Gun. 1952: 17, Rad. 1954: 250.
Trombicula (*Trombicula*) *densipiliata*, W. & F. 1952: 64.
Trombicula (*vorca*-group) *densipiliata*, Audy 1954*b*: 148 (t).
Trombicula (*Vorcana*) *densipiliata*, Audy 1956*b*: 52 (tx).
Trombicula (*Vorcana*) *densipiliata densipiliata* Wom. & A. 1957 (dxl).

Note. Described from one specimen found unattached on the ground, not from 'Rat' (Gun. 1952: 17) or '*Rattus* sp.' (Rad. 1954: 250).

(2) **Trombicula (Vorcana) densipiliata dumbletoni** Womersley & Audy
Trombicula cf. *densipiliata*, Wom. 1952: 126 (23) (specimen from Nissan, Solomon Is.).
Trombicula (*Vorcana*) *densipiliata dumbletoni* Wom. & A., 1957: ... (Dxl).
Records. OCEANIA, Solomon Is. Rodent—'Rat'.

Trombicula (Vorcana) nissani Dumbleton

Trombicula nissani, Wom. 1952: 113 (18) d, Gun. 1952: 20, Traub & Audy 1954*a*: 48 (t), Audy 1954*b*: 148 (t), Rad. 1954: 258.
Trombicula (*Trombicula*) *nissani*, W. & F. 1952: 68.
Trombicula (? *Vorcana*) *nissani*, Audy 1956*b*: 52 (t), Wom. & A. 1957 (xl).

Trombicula (Vorcana) samara Radford, new status

Trombicula samara Rad., 1953*b*: 242 (D), 1954: 258.
Records. PHILIPPINES. Bird—*Centropus melanops.*

Trombicula (Vorcana) vorca* Traub & Audy

Trombicula vorca Traub & A., 1954*a*: 47 (56, 57) D, Audy 1954*b*: 144 (t).
Trombicula (*Vorcana*) *vorca*, Audy 1956*b*: 52, 59 (ct).
Records. BORNEO. Bird—Unidentified chick.

Subgenus uncertain

Trombicula anous† Wharton

Trombicula anous, Wom. 1952: 98 (14) d, Gun. 1952: 20, Kano & Sasa 1952: 15, Sasa & Jam. 1954: 312 (313) dc, Audy 1954*b*: 148 (t), Wom. & A. 1957 (l).
Trombicula (*Trombicula*) *anous*, W. & F. 1952: 62.
Eutrombicula (*sic*!) *anous*, Rad. 1954: 261.

Trombicula cervulicola Ewing

Trombicula cervulicola, Wom. 1952: 125 (22) d, Gun. 1952: 19, Rad. 1954: 250, Wom. & A. 1957 (l).
Trombicula (*Trombicula*) *cervulicola*, W. & F. 1952: 63.

Trombicula consueta Womersley

Trombicula (*Neotrombicula*) *consueta* Wom., 1952: 108 (110) D, 351 (kN) 402 (l).
Trombicula consueta, Audy 1954*b*: 143 (t), 148, 1956*d*: 94 (ct), Har. 1957 (b), Wom. & A. 1957: ... (DN).
Neotrombicula consueta, Rad. 1954: 259.
Records. MALAYA. Rodent—*Rattus bowersi*.

Trombicula flagellifera‡ Traub & Audy

Trombicula flagellifera Traub & A., 1954: 46 (52, 53) (D), Audy 1954*b*: 144 (t).
Records. BORNEO. Rodent—*Nannosciurus whiteheadi*. Insectivores—*Crocidura* sp., *Hylomys suillus*.

Trombicula (?) geckobia§ (Womersley)

Tragardhula geckobia Wom., 1952: 29 (2) D, Rad. 1954: 262.
Trombicula geckobia, Audy 1954*b*: 145, 148 (t), 1956*b*: 54, 55 (t), Wom. & A. 1957 (xl).
Records. AUSTRALIA. Reptile—Gecko.

* This species, or forms extremely close to it, have also been recorded by Traub & Evans 1954: 48, and Audy 1956*b*: 59, as follows: BORNEO. Birds—*Centropus* sp. MALAYA. Birds—*Halcyon c. concreta, Otus bakkamoena lempiji*. Nymphs have been reared and both forms are being studied.

† Species of *T. anous*, *T. pluvius* and *T. shiraii* Sasa *et al.* form the *anous*-group of Audy (1954*b*). *T. anous* is now known from a bird (*Pluvialis dominicus*) from Japan.

‡ Species of *T. flagellifera* and *T. tuhana* were placed in a *flagellifera*-group by Audy (1954*b*), but his suggestion that they may be related to *Neotrombicula* is dubious.

§ The *geckobia*-group of Audy (1954*b*: 145, 148) requires rearrangement as follows: (i) *geckobia* is distinct but might be related to the others, (ii) *incurva* is an *Eutrombicula* s.l., (iii) *gymnodactyla*, *naultini* and *pentagona* might be grouped together provisionally.

Trombicula (?) gymnodactyla* Womersley & Kohls

Tragardhula gymnodactyla, Wom. 1952: 30 (2) d, Rad. 1954: 262.
Trombicula (*Trombicula*) *gymnodactyla*, W. & F. 1952: 65.
Trombicula (*geckobia*-group) *gymnodactyla*, Audy 1954*b*: 145, 148 (t).
Eutrombicula gymnodactyla, Rad. 1954: 261.
Trombicula (?) *gymnodactyla*, Wom. & A. 1957 (xl).
Records. AUSTRALIA. Reptiles—'Gecko', *Phyllodactylus marmoratus*.

Trombicula leveri† Womersley

Trombicula leveri Wom., 1952: 131 (24) D, Audy 1952: 146 (k), 1954*b*: 148 (t), 1956*d*: 94 (c), Rad. 1954: 257, Wom. & A. 1957 (xl).
Records. OCEANIA, Fiji. Bat—*Emballonura* sp.

Trombicula (?) naultini Dumbleton

Trombicula naultini, Wom. 1952: 124 (22) d, Gun. 1952: 22, Rad. 1954: 258.
Trombicula (? *geckobia*-group) *naultini*, Audy 1954*b*: 148 (t).
Trombicula (*Trombicula*) *naultini*, W. & F. 1952: 68.

Trombicula (?) pentagona (Womersley)

Tragardhula pentagona Wom., 1952: 29 (2) D, Rad. 1954: 262.
Trombicula (?) *pentagona*, Wom. & A. 1957 (xl).
Records. AUSTRALIA. Unattached. Arthropod—*Troides priamus* (Lepidoptera) (single straggler?).

Trombicula philipi‡ Womersley

Trombicula philipi Wom., 1952: 135 (25) D, Rad. 1954: 258, Wom. & A. 1957 (l).
Trombicula (*muscae*-group) *philipi*, Audy 1954*b*: 148 (t).
Records. NEW GUINEA. Bats—*Hipposideros cervinus*.

Trombicula piercei Ewing

Trombicula piercei, Wom. 1952: 123 (24) d, Audy 1952: 146 (k), Gun. 1952: 19, Loomis 1954: 924, Rad. 1954: 258, Wom. & A. 1957 (xl).
Trombicula (*Trombicula*) *piercei*, W. & F. 1952: 68, Audy 1954*b*: 147 (t).

Trombicula pluvius Wharton

Trombicula pluvius, Wom. 1952: 99 (4) d, Gun. 1952: 20, Wom. & A. 1957 (l).
Trombicula (*Trombicula*) *pluvius*, W. & F. 1952: 69.
Trombicula (*anous*-group) *pluvius*, Audy 1954*b*: 148 (t).
Eutrombicula (*sic*!) *pluvius*, Rad. 1954: 261.

* See footnote § on p. 21. † Placed in a group with *T. revelae* by Audy 1954*b*: 148.
‡ The *muscae*-group, in which Audy (1954*b*: 148) tentatively placed *T. muscae*, *T. philipi*, *T. quadriense* (definitely not in this group), and *T. taphozous*, requires further elucidation. *T. philipi* and *T. taphozous* form a group which is apparently related to the *anous*-group.

Trombicula quadriense Womersley & Heaslip

Trombicula quadriense, Wom. 1952: 136 (25) ds, Audy 1952: 146 (k), Gun. 1952: 22, Rad. 1954: 258, Wom. & A. 1957 (xl).

Trombicula chiroptera, Gun. 1952: 22, Rad. 1954: 250.

Trombicula (*Trombicula*) *quadriense*, W. & F. 1952: 69.

Trombicula (*muscae*-group)(!) *quadriense*, Audy 1954*b*: 148, wrong group.

Records. AUSTRALIA. Marsupials—'Bandicoot' (Peramelidae), 'Possum' (Phalangeridae). Bats ('possibly from South Australia', for *T. chiroptera*)—*Chalinolobus gouldii*; and unnamed, unlocalized bats. OCEANIA, Solomons (?). ? Host.

Trombicula revelae Audy

Trombicula revelae Audy, 1952: 140 (D), 1956*d*: 94 (c).

Records. MALAYA. Bats—*Hipposideros* sp.

Trombicula southcotti Womersley

Trombicula southcotti Wom., 1952: 138 (26) D, Rad. 1954: 258, Wom. & A. 1957 (xl).

Records. AUSTRALIA. Marsupial—'Bandicoot'.

Trombicula taphozous* Womersley

Trombicula taphozous Wom., 1952: 122 (21) D, Audy 1952: 144 (dc), 147 (k), Rad. 1954: 258, Wom. & A. 1957 (xlt).

Trombicula (*muscae*-group) *taphozous*, Audy 1954*b*: 148 (t), 1956*d*: 94 (c).

Records. MALAYA. Bats—*Hipposideros* sp., *Taphozous melanopogon*.

Trombicula tuhana Traub & Audy

Trombicula tuhana Traub & A., 1954*a*: 46 (54, 55) D, Audy 1954*b*: 144.

Records. BORNEO. Rodent—*Nannosciurus whiteheadi*.

Trombicula (?) victoriensis Audy & Womersley

Trombicula (?) *victoriensis* Audy & Wom., 1957: ... (DA).

Records. AUSTRALIA. Free-living adult.

Trombicula vietzi Womersley

Trombicula vietzi Wom., 1952: 130 (110) D, Rad. 1954: 258, Wom. & A. 1957 (xl).

Records. INDIA, Peninsular. Rodent—'Rat'.

* See footnote to *T. philipi*.

Genus **Tecomatlana** Hoffman, 1947

Type: *Tecomatlana sandovali* Hoffman, 1947

Subgenus **Trisetica** Traub & Evans, 1950

Type: *Trisetica melvini* Traub & Evans, 1950

Tecomatlana (Trisetica) melvini Traub & Evans

Tecomatlana melvini, Wom. 1952: 147 (29) d, W. & F. 1952: 90.
Trisetica melvini, Gun. 1952: 37, Rad. 1954: 267.
Tecomatlana (*Trisetica*) *melvini*, Audy 1954*b*: 149 (t), Wom. & A. 1957 (xl).

New genus, Vercammen-Grandjean & Brennan

Type: New species from bat, Kenya

'Trombicula' dimolinae Audy

Trombicula dimolinae Audy, 1952: 136 (D), Wom. & A. 1957 (xl).
Trombicula (*Trombicula*) *dimolinae*, Audy 1954*b*: 147 (tx), 1956*d*: 92 (cd).
Records. MALAYA. Bat—*Rhinolophus* sp.

'Trombicula' harrisoni Womersley

Trombicula (*Neotrombicula*) *harrisoni* Wom. 1952: 144 (29) D, 372 (113) DN, Audy 1952: 135 (c), 147 (k).
Trombicula (*Trombicula*) *harrisoni*, Audy 1954*b*: 147 (t), 1956*d*: 92 (cd).
Trombicula harrisoni, Rad. 1954: 257, Wom. & A. 1957 (xl).
Neotrombicula harrisoni, Rad. 1954: 259.
Records. MALAYA. Bats—*Hipposideros* spp.

'Euschongastia' lipoxena (Womersley)

Schongastia (*Ascoschongastia*) *lipoxena* Wom., 1952: 199 (52) D.
Euschongastia lipoxena, Audy 1952: 148 (143) dc, 1954*b*: 155, 1956*d*: 97 (dc), Rad. 1954: 265, Wom. & A. 1957 (xl).
Records. MALAYA. Bats—*Hipposideros* spp.

Genus **Eutrombicula** Ewing, 1938

Type: *Microthrombidium alfreddugesi* Oudemans 1910

Subgenus **Eltonella** Audy, 1956

Type: *Eutrombicula* (*Eltonella*) *eltoni* Audy, 1956

Eutrombicula (Eltonella) eltoni Audy

'*Trombicula* (*Tragardhula*?) sp. indet.', Audy 1950 (c).
Trombicula (*Trombicula*) *frittsi*, in part, Wom. 1952: 128 (23) d, 330 (87) DN, mis-identification.

Trombicula (*?ilesi*-group) *frittsi*, in part, Audy 1954*b*: 146, 148 (t), mis-identification.

Eutrombicula (*Eltonella*) *eltoni* Audy, 1956*b*: 32 (D), 1956*d*: 94 (c), Wom. & A. 1957 (xl).

Records. MALAYA. Arthropod (Scorpionida)—*Heterometrus longimanus.*

Eutrombicula (Eltonella) frittsi (Wharton)

Trombicula (*Trombicula*) *frittsi*, in part, Wom. 1952: 128 (23) d (*not* 330 (87) DN, confused with *eltoni*).

Trombicula (*Trombicula*) *frittsi*, W. & F. 1952: 65.

Trombicula frittsi, Gun. 1952: 20, Rad. 1954: 250.

Trombicula (*? ilesi*-group) *frittsi*, in part, Audy 1954*b*: 146, 148 (t).

Eutrombicula (*Eltonella*) *frittsi*, Audy 1956*b*: 36 (34, 35) dt, Wom. & A. 1957 (xl).

Eutrombicula (Eltonella) tweediei Audy

Eutrombicula (*Eltonella*) *tweediei* Audy, 1956*b*: 36 (D), 1956*d*: 94 (c).

Records. MALAYA. Reptiles—*Draco blandfordi*, *D. fimbriatus*, *D. melanopogon*, *D. quinquefasciatus*, *D. volans.*

Subgenus **Eutrombicula** Ewing, 1938

Type: *Microthrombidium alfreddugesi* Oudemans, 1910

Eutrombicula (Eutrombicula) ablephara (Womersley)

Trombicula ablephara Wom., 1952: 86 (14) D, Rad. 1954: 250.

Eutrombicula (*Eutrombicula*) *ablephara*, Audy 1956*b*: 31 (tx), Wom. & A. 1957 (xl).

Records. NEW GUINEA. Reptile—*Ablepharus boutoni* (series from skink *Lygosoma* (*Liolepisma*) *challengeri* from AUSTRALIA, noted by Womersley, may be a distinct species).

Eutrombicula (Eutrombicula) hirsti (Sambon)

Trombicula (*Trombicula*) *hirsti*, Wom. 1952: 82 (13) d.

Trombicula hirsti, Gun. 1952: 7.

Trombicula (*Eutrombicula*) *hirsti*, W. & F. 1952: 47, Audy 1954*b*: 146 (t), Hammen 1956 (dm).

Eutrombicula hakei, Rad. 1954: 261.

Eutrombicula hirsti, Rad. 1954: 261.

Eutrombicula (*Eutrombicula*) *hirsti*, Audy 1956*b*: ... (t), Wom. & A. 1957 (xl).

Records. INDIA, North-east. Reptile—*Coluber radiatus* (for *E. hakei* syn.). NEW GUINEA. Mammals—Pigs, man (for the form *nissanense* Wom.).

Eutrombicula (Eutrombicula) isshikii Sugimoto

Trombicula isshikii, Wom. 1952: 113 (18) d, Gun. 1952: 14.

Trombicula (*Eutrombicula*) *isshikii*, W. & F. 1952: 48, Audy 1954*b*: 146.

Eutrombicula isshikii, Rad. 1954: 261.

Eutrombicula (Eutrombicula) kohlsi (Womersley)

Trombicula (? *Neotrombicula*) *kohlsi*, Wom. 1952: 116 (19) d.
Trombicula kohlsi, Gun. 1952: 20.
Trombicula (*Eutrombicula*) *kohlsi*, W. & F. 1952: 48, Audy 1954*b*: 146 (t).
Eutrombicula (*Eutrombicula*) *kohlsi*, Audy 1956*b* (tx), Wom. & A. 1957 (d).
Neotrombicula kohlsi, Rad. 1954: 259.

Eutrombicula (Eutrombicula) macropus (Womersley)

Trombicula macropus, Wom. 1952: 103 (16) d, Gun. 1952: 22, Rad. 1954: 257.
Trombicula (*Eutrombicula*) *macropus*, W. & F. 1952: 49, Audy, 1954*b*: 146 (t).
Eutrombicula (*Eutrombicula*) *macropus*, Audy 1956*b* (tx), Wom. & A. 1957 (xl).

Eutrombicula (Eutrombicula) rioi (Gunther)

Trombicula rioi, Wom. 1952: 104 (16) d, Gun. 1952: 22.
Trombicula (*Eutrombicula*) *rioi*, W. & F. 1952: 49.
Eutrombicula rioi, Rad. 1954: 261.
Eutrombicula (*Eutrombicula*) *rioi*, Audy 1956*b* (tx), Wom. & A. 1957 (xl).
Records. NEW GUINEA. Bird—Nest of bush-turkey (*Macropus*).

Eutrombicula (Eutrombicula) samboni (Womersley)*

Trombicula (*Trombicula*) *samboni*, Wom. 1952: 101 (15) d, 349 (89) dNA.
Trombicula (*Eutrombicula*) *samboni*, W. & F. 1952: 81, Audy 1954*b*: 144, 146 (t).
Trombicula samboni, Gun. 1952: 21, Rad. 1954: 258.
Eutrombicula (*Eutrombicula*) *samboni*, Audy 1956*b* (tx), Wom. & A. 1957 (xl).

Eutrombicula (Eutrombicula) sarcina (Womersley)

Trombicula (*Neotrombicula*) *sarcina*, Wom. 1952: 101 (15) d, 362 (97) DN.
Trombicula sarcina, Gun. 1952: 22.
Trombicula (*Eutrombicula*) *sarcina*, W. & F. 1952: 50, Audy 1954*b*: 144, 146 (t).
Neotrombicula sarcina, Rad. 1954: 259.
Eutrombicula (*Eutrombicula*) *sarcina*, Audy 1956*b* (tx), Wom. & A. 1957 (xl).

Eutrombicula (Eutrombicula) scincoides (Womersley)

Trombicula (*Neotrombicula*) *scincoides*, Wom. 1952: 114 (19) d, 351 (93) DNA.
Trombicula (*Trombicula*) *scincoides*, W. & F. 1952: 70.
Trombicula scincoides, Gun. 1952: 20.
Trombicula (*Eutrombicula*) *scincoides*, Audy 1954*b*: 146.
Neotrombicula scincoides, Rad. 1954: 259.
Eutrombicula (*Eutrombicula*) *scincoides*, Audy 1956*b* (tx), Wom. & A. 1957 (xl).
Records. NEW GUINEA. Reptiles—'Gecko', 'Skinks'. PHILIPPINES. Reptile—'Mottled Skink'.

* This localized species may prove to be a subspecies of the tropical Australian *E.* (*E.*) *hirsti*.

Eutrombicula (Eutrombicula) sobrina (Womersley)

Trombicula sobrina Wom., 1952: 85 (13) D, Rad. 1954: 258.
Trombicula (*Eutrombicula*) *sobrina*, Audy 1954*b*: 146 (t).
Eutrombicula (*Eutrombicula*) *sobrina*, Audy 1956*b* (tx), Wom. & A. 1957 (xl).
Records. NEW GUINEA. Reptile—*Lygosoma stanleyanum*.

Eutrombicula (Eutrombicula) tovelli (Womersley)

Trombicula sp. 'common on skinks', in part, Southcott 1947: 450.
Trombicula (? *Neotrombicula*) *tovelli*, Wom. 1952: 116 (19) D.
Trombicula (*Eutrombicula*) *tovelli*, Audy 1954*b*: 146 (t).
Neotrombicula tovelli, Rad. 1954: 260.
Eutrombicula (*Eutrombicula*) *tovelli*, Audy 1956*b*: 31 (tx), Wom. & A. 1957 (xl).
Records. AUSTRALIA. Reptiles—'Lizard' 'Skinks', *Sphenomorphus taeniolatus*.

Eutrombicula (Eutrombicula) wichmanni (Oudemans)

Trombicula (*Trombicula*) *wichmanni*, Wom. 1952: 87 (14) d, 336 (89) dNA.
Trombicula (*Eutrombicula*) *wichmanni*, W. & F. 1952: 50, Sasa & Jam. 1952: 310 (331) dc, Sasa 1953: 421 (441) dNA, Audy 1954*b*: 146 (t), Harrison 1954: 180 (b), 1956 (c), 1957 (b), Hammen 1956 (dm).
Eutrombicula wichmanni, Fuller 1952: 117 (dtcm).
Trombicula wichmanni, IMR 1949: 64 (bc), Gun. 1952: 8, Rad. 1954: 258, Suy. & Tosh. 1955 (cl).
Trombicula pseudoakamushi, Gun. 1952: 12.
Eutrombicula (*Eutrombicula*) *wichmanni*, Audy 1956*b*: 59, 73 (ctx), 1956*d*: 94 (c), Wom. & A. 1957 (xl), Har. 1957*a* (c).
Records. MALAYA. Birds—*Gallus g. domesticus*, *Turnix suscitator atrogularis*. Reptiles—*Draco volans*, *Ptyas korros*, *Mabuya multifasciata* (chief host), *Varanus dumerilii*, *V. flavescens*. BORNEO. Unattached. CELEBES. Unattached. Primate —'On man'. NEW GUINEA. Reptiles—*Lygosoma* sp., 'snake'. PHILIPPINES. Primate—'On man'.

Subgenus uncertain*

Eutrombicula fieldi Audy

Eutrombicula (*fieldi*-group) *fieldi* Audy, 1957*b*: 38 (Dt), 1956*d*: 94 (c).
Records. MALAYA. Reptiles—*Lygosoma olivaceum*, *Draco volans*.

Eutrombicula incurva (Womersley)

Trombicula incurva Wom., 1952: 130 (24) D, Rad. 1954: 257.
Trombicula (*geckobia*-group) *incurva*, Audy 1954*b*: 146, 148 (t).
Eutrombicula incurva, Wom. & A. 1957 (xl).
Records. AUSTRALIA. Reptile—*Peropus variegatus*.

* The three species here listed appear to link *Eutrombicula* to the African *lawrencei*-group through the *fieldi*-group (*fieldi*, *lygosomoides*) of Audy (1956*b*: 38).

Eutrombicula lygosomoides (Womersley)

Trombicula lygosomoides Wom., 1952: 100 (D), Rad. 1954: 257.

Trombicula (*Eutrombicula*) *lygosomoides*, Audy 1954*b*: 146 (t).

Eutrombicula (*fieldi*-group) *lygosomoides*, Audy 1956*b*: 38 (dt), Wom. & A. 1957 (xl).

Records. AUSTRALIA. Reptile—*Lygosoma stanleyanum*.

Genus **Fonsecia** Radford, 1946

Type: *Trombicula ewingi* Fonseca, 1932

Fonsecia celesteae Audy

Fonsecia celesteae Audy 1956*b*: 49 (D), 1956*d*: 95 (c).

Records. MALAYA. Reptiles—*Mabuya multifasciata*, *Natrix trianguligera*.

Fonsecia coluberina Radford

Trombicula coluberina, Wom. 1952: 104 (16) d, Gun. 1952: 19.

Fonsecia coluberina, Audy 1954*b*: 148 (t), 1956*b*: 51 (dt), Rad. 1954: 262, Wom. & A. 1957 (xl).

Genus **Ipotrombicula** Womersley, 1952

Type: *Trombicula elegans* Womersley, 1942

Ipotrombicula elegans (Womersley)

Ipotrombicula elegans, Wom. 1952: 323 (86) dA, Audy 1954*b*: 150, 1956*b*: 52, 56 (tc), Wom. & A. 1957 (l).

Trombicula (*Trombicula*) *elegans*, W. & F. 1952: 65.

Note. Hosts unknown, but probably birds or lizards.

Genus **Vercammenia** Audy & Nadchatram, 1957

Type: *Vercammenia hendricksoni* Audy & Nadchatram 1957

Vercammenia hendricksoni Audy & Nadchatram

Vercammenia hendricksoni Audy & Nad., 1957*b*: ... (D).

Records. MALAYA. Amphibians—*Megophrys hasseltii*, *Rana* sp. nr. *macrodon* (chief host), *R. s. signata*.

Genus **Blankaartia** Oudemans, 1911*

Tragardhula Berlese, 1912

Type: *Trombidium niloticum* Tragardh, 1904 (adult)

Blankaartia [Tragardhula] acuscutellaris (Walch)

Tragardhula acuscutellaris, Wom. 1952: 24 (1) d, 316 (84) dNA, Rad. 1954: 262.
Trombicula acuscutellaris, Gun. 1952: 17, Fuller 1952: 80 (dtcm).
Trombicula (*Blankaartia*) *acuscutellaris*, W. & F. 1952: 43, Audy 1954*b*: 145.
Blankaartia acuscutellaris, Harrison 1954: 180 (b), Audy 1956*b*: 62, 78 (ctx), 1956*d*: 95 (cd).
Blankaartia [*Tragardhula*] *acuscutellaris*, Verc. & A. 1957 (tc), Har. 1957 (b).
Tragardhula [*Blankaartia*] *acuscutellaris*, Wom. & A. 1957 (txl).
Records.† MALAYA. Rodents—*Rattus argentiventer*. Birds—*Amaurornis phoenicurus chinensis*, *Capella stenura*, *Centropus* sp., *Drymophila p. pyrhoptera*, *Ixobrychus eurythmus*.

Genus **Heaslipia**‡ Womersley, 1942

Type: *Trombiculoides gateri* Womersley & Heaslip, 1943

Heaslipia gateri (Womersley & Heaslip)

(1) **Heaslipia gateri gateri** (Womersley & Heaslip)
Heaslipia gateri, Wom. 1952: 149 (30) d, 422 (113, 116) DN, Gun. 1952: 37, W. & F. 1952: 83, Audy 1954*b*: 149, 1956*b*: 62, 78 (ct), 1956*d*: 95 (c), Rad. 1954: 262.
Heaslipia gateri gateri, Verc. & A. 1957: ... (dtc, dN).
Records. MALAYA. Bird—*Ixobrychus eurythmus*. PHILIPPINES. Rodent—'Rat'.

(2) **Heaslipia gateri africana** Vercammen-Grandjean & Audy
Recorded from AFRICA on the water-bird, *Centropus grilli*.

Genus **Neotrombicula** Hirst, 1915

Type: *Acarus autumnalis* Shaw, 1790

Neotrombicula anax Audy & Womersley

Trombicula (*Neotrombicula*) '*AX*', Audy 1954*b*: 145.
Neotrombicula anax Audy & Wom., 1957: ... (D).
Records. AUSTRALIA. North. Rodents—'Rats'.

* *Trombidium niloticum*, the type species, is a confusion of two species (adult trombiculid and larval trombidiid) and the genus to which the adult trombiculid is to be ascribed is at present being considered by the International Commission on Zoological Nomenclature. *Blankaartia* is used here because this list is in conformity with that of Wharton & Fuller.

† The previous records for INDIAN OCEAN, Maldives (Rad. 1946: 48) should probably be for *Rattus r. alexandrinus* (not *R. n. norvegicus, err. det.*).

‡ The post-larval stages of *Heaslipia* have paracristal eyes and are indistinguishable by present criteria from post-larval stages of *Blankaartia* [*Tragardhula*]. The primary hosts of the larvae of *Heaslipia* appear to be water-birds, as with the larvae of *Blankaartia* [*Tragardhula*].

Neotrombicula antechinus (Womersley), new combination

Trombicula antechinus Wom. 1954: 69 (D).

Records. AUSTRALIA. Marsupial—*Antechinus flavipes*.

Neotrombicula derricki (Womersley), new combination

Trombicula derricki Wom., 1954: 67 (D).

Records. AUSTRALIA. Unattached.

Neotrombicula fujigmo (Philip & Fuller)

Tragardhula fujigmo, Wom. 1952: 28 (2) d, Audy *et al.* 1953: 34 (l), Rad. 1954: 262.

Trombicula fujigmo, Gun. 1952: 14.

Trombicula (*Neotrombicula*) *fujigmo*, W. & F. 1952: 58, Audy 1954*b*: 145.

Neotrombicula fujigmo, Wom. & A. 1957 (xl).

Records (identity of mite requiring confirmation). INDIA, North-east. Rodent—*Rattus r. bullocki*.

Neotrombicula kanzalwanensis (Womersley)

Trombicula kanzalwanensis Wom., 1952: 102 (21) D, Rad. 1954: 257.

Trombicula (*Neotrombicula*) *kanzalwanensis*, Audy 1954*b*: 145 (t).

Neotrombicula kanzalwanensis, Wom. & A. 1957 (dxl).

Records. INDIA, North. Rodents—*Apodemus flavicollis*, 'Rats'.

Neotrombicula kashmirensis (Womersley)

Trombicula kashmirensis Wom., 1952: 120 (20) D.

Trombicula kashmirensis, Rad. 1954: 257.

Trombicula (*Neotrombicula*) *kashmirensis*, Audy 1954*b*: 145 (t).

Neotrombicula kashmirensis, Wom. & A. 1958 (dxl).

Records. INDIA, North. Rodents—*Golunda ellioti*, 'Rat'.

Neotrombicula mackayensis (Womersley), new combination

Trombicula mackayensis, Wom. 1954: 72 (D).

Records. AUSTRALIA. Unattached.

Neotrombicula novae-hollandiae (Hirst)

Trombicula novae-hollandiae, Wom. 1952: 79 (12) d, 1954: 69, Gun. 1952: 21, Rad. 1954: 258.

Trombicula (*Trombicula*) *novae-hollandiae*, W. & F. 1952: 68.

(?) *Neoschongastia novae-hollandiae* Wharton, 1946 (*sic*!), ex *Rattus greyi*, Rad. 1954: 263, *nom. nud.*

Neotrombicula novae-hollandiae, Wom. & A. 1957: ... (dxl).

Neotrombicula thylogale (Womersley), new combination

Trombicula thylogale, Wom. 1954: 71 (D).

Records. AUSTRALIA. Marsupial—*Thylogale wilcoxi*.

Genus **Siseca** Audy, 1956

Type: *Trombicula rara* Walch, 1923

Siseca lundbladi (Womersley)

Trombicula lundbladi Wom., 1952: 110 (18) D, Rad. 1954: 257.

Trombicula (*parmifera*-group)(!) *lundbladi*, Audy 1954*b*: 148 (t), wrong group.

Siseca lundbladi, Audy 1956*b*: 42 (44) dt, Wom. & A. 1957 (xl).

Records. NEW GUINEA. Reptile—'Dark green and striped skink'.

Siseca rara (Walch)

Trombicula (*Neotrombicula*) *rara*, Wom. 1952: 80 (13) d (in part), 427 (x).

Trombicula rara, Gun. 1952: 17.

Trombicula (*Trombicula*) *rara*, W. & F. 1952: 69.

Trombicula (*rara*-group) *rara*, Audy 1954*b*: 143, 148 (t).

Neotrombicula rara, Rad. 1954: 259.

Siseca rara, Audy 1956*b*: 42, 60 (44) dc, 1956*d*: 95 (ct), Har. 1957 (b), Wom. & A. 1957 (dxl).

Records. MALAYA. Rodents (casual)—*Callosciurus* spp. *Rattus* spp. Insectivores (casual)—*Tupaia* spp. Reptile—*Mabuya multifasciata* (chief host). *Corrigendum*: *Not* from arthropod, ? *Zephronia*, Audy 1950, W. & F. 1952: 69, 138, nor from *Sphaeropaeus*, Wom. 1952: 355 (93) DN (refer to *subrara*).

Siseca southcotti Womersley & Audy

Trombicula (*Neotrombicula*) *rara*, in part, Wom. 1952: 81 (c), *err. det.*

Siseca southcotti Wom. & A., 1957: ... (D).

Records. AUSTRALIA. Reptiles—*Lygosoma* (*Liolepisma*) *rhomboidalis*.

Siseca subrara Audy

Trombicula rara, Audy 1950: 193 (c), *err. det.*

Trombicula (*Neotrombicula*) *rara*, ex millipedes, Wom. 1952: 80 (13) d in part, 355 (93) DN.

Trombicula (*rara*-group), n.sp. nr. *rara*, Audy 1954*b*: 148.

Siseca subrara Audy 1956*b*: 42 (D), 1956*d*: 95 (c), Wom. & A. 1957 (dxl).

Records. MALAYA. Arthropod (oniscomorph diplopod)—*Sphaeropaeus globus-magicus*; '? other pill-millipedes'.

Siseca thori (Womersley)

Trombicula thori Wom., 1952: 421 (116) D, Rad. 1954: 258.
Trombicula (? *rara*-group) *thori*, Audy 1954*b*: 148 (t).
Siseca thori, Audy 1956*b*: 42 (tx), Wom. & A. 1957 (xl).
Records. AUSTRALIA. Reptile—*Lygosoma* (*Liolepisma*) *melanopogon.*

Genus **Babiangia** Southcott, 1954

Type: *Babiangia bulbifera* Southcott, 1954

Babiangia booliati Audy

'*Trombicula* (*parmifera*-group) "*MAB*" n.sp.', Audy 1954*b*: 148 (t).
Babiangia booliati Audy, 1956*b*: 46 (D).
Records. MALAYA. Reptile—*Mabuya multifasciata.*

Babiangia bulbifera Southcott

Babiangia bulbifera Southcott, 1954: 98 (D), Audy 1956*b*: 54 (t).
Records. NEW GUINEA. Unattached. Reptile—*Lygosoma* (*Homolepida*) *forbesii*?.

Babiangia parmifera (Womersley)

Trombicula parmifera Wom., 1952: 109 (17) D, 2c (x), Audy 1954*b*: 148 (t), Rad. 1954: 258, Harrison 1954: 180 (b).
Babiangia parmifera, Audy 1956*b*: 60 (47, 48) dtc, 1956*d*: 95 (dc), Har. 1957 (b), Wom. & A. 1957: ... (xl, dN).
Records. MALAYA. Reptiles—*Lygosoma bowringi*, *Mabuya multifasciata* (chief host).

Genus **Novotrombicula** Womersley & Kohls, 1947

Type: *Novotrombicula owiensis* Womersley & Kohls, 1947

Novotrombicula owiensis Womersley & Kohls

Novotrombicula owiensis, Wom. 1952: 151 (31) d, Gun. 1952: 37, W. & F. 1952: 87, Audy 1954*b*: 149 (t), 1956*b*: 59 (t), Rad. 1952: 262, Wom. & A. 1957 (xl).

Genus **Myotrombicula** Womersley & Heaslip, 1943

Type: *Myotrombicula vespertilionis* Womersley & Heaslip, 1943

Myotrombicula vespertilionis Womersley & Heaslip

Myotrombicula vespertilionis, Wom. 1952: 148 (30) d, W. & F. 1952: 84, Gun. 1952: 136, Audy 1954*b*: 149 (t), Rad. 1954: 267, Wom. & A. 1957 (xl).

Genus **Riedlinia** Oudemans, 1914

Type: *Riedlinia coeca* Oudemans, 1914

Riedlinia coeca Oudemans

Riedlinia coeca, Fuller 1952: 199 (dt), W. & F. 1952: 87, Audy 1954*b*: 160 (tx), Wom. & A. 1957 (xl).

Reidlinia (*sic*!) *coeca*, Wom. 1952: 21 (k), Rad. 1954: 262.

Note. Locality is INDIA, near Bombay, *not* Malaya.

Genus **Schongastia*** Oudemans, 1910

Type: *Thrombidium vandersandei* Oudemans, 1905

Schongastia bidentata Womersley

Schongastia (*Schongastia*) *bidentata* Wom., 1952: 163 (34) D.

Euschongastia bidentata, Audy 1954*b*: 159 (t).

Schongastia bidentata, Rad. 1954: 266, Verc. 1956 (t), Audy & Dom. 1957 (t), Wom. & A. 1957 (xl).

Records. AUSTRALIA. Reptile—'Skink'.

Schongastia blestowei† Gunther (? synonym)

Schongastia (*Schongastia*) *blestowei*, Wom. 1952: 165 (36) d.

Schongastia (*Schongastia*) *blestowei* form *megapodius*, Wom. 1952: 166 (36) d.

Schongastia vandersandei, W. & F. 1952: 88 (s), Fuller 1952: 157, 152 (ts).

Schongastia blestowei, Gun. 1952: 24, Rad. 1954: 266.

Schongastia blestowei (? syn.), Wom. & A. 1957 (xl).

Records. NEW GUINEA. Unattached. Bird (?)—Nest of *Megapodius*. OCEANIA. Primate—'On Man'.

Schongastia edwardsi (Gunther)

Schongastia (*Ascoschongastia*) *edwardsi*, Wom. 1952: 181 (44) d.

Neoschongastia edwardsi, Gun. 1952: 29.

Euschongastia edwardsi, W. & F. 1952: 76, Audy 1954*b*: 155, Rad. 1954: 265.

Schongastia (*?*) *edwardsi*, Verc. 1956*a* (tx), Wom. & A. 1957 (txl), Audy & Dom. 1957.

Schongastia jamesi Gunther

Schongastia jamesi, W. & F. 1952: 88, Gun. 1952: 25, Rad. 1954: 26, Wom. & A. 1957 (xl).

* The following species was described after this checklist went to press: *Schongastia propria* Audy & Wom., 1957. INDIA, Peninsular. Reptile—*Coluber karelini* (?).

† *S. blestowei* appears to be a synonym of *S. vandersandei*, but until differences of opinion are resolved it is left as a species *sub judice*.

Schongastia philipi Womersley & Kohls

Schongastia (*Schongastia*) *philipi*, Wom. 1952: 164 (35) d.

Schongastia philipi, Gun. 1952: 25, W. & F. 1952: 89, Rad. 1957: 267, Wom. & A. 1957 (xl).

Schongastia pseudoschuffneri (Walch)

Schongastia (*Schongastia*) *pseudoschuffneri*, Wom. 1952: 167 (37) d.

Schongastia pseudoschuffneri, Gun. 1952: 24, W. & F. 1952: 89, Audy *et al.* 1953: 34, Rad. 1954: 267, Wom. & A. 1957 (xl).

Records. BURMA, South. Rodent—*Bandicota bengalensis.* INDIA, North-east. Rodent—*Rattus r. bullocki.*

Schongastia schuffneri (Walch)

Schongastia (*Schongastia*) *schuffneri*, Wom. 1952: 159 (33) d.

Schongastia schuffneri, Gun. 1952: 24, W. & F. 1952: 89, Fuller 1952: 164 (dtm), Rad. 1952: 267, Hammen 1956 (dm), Wom. & A. 1957 (xl).

Schongastia katonis, W. & F. 1952: 88, Gun. 1952: 25, Rad. 1954: 266.

Records. CELEBES and NEW GUINEA. No host data.

Schongastia taylori Gunther

Schongastia (*Schongastia*) *taylori*, Wom. 1952: 160 (33) d.

Schongastia taylori, Gun. 1952: 25, W. & F. 1952: 88, Rad. 1954: 267, Wom. & A. 1957 (xl).

Schongastia vandersandei (Oudemans)

Schongastia (*Schongastia*) *vandersandei*, Wom. 1952: 155 (32) d.

Schongastia vandersandei, Gun. 1952: 23, W. & F. 1952: 87, Fuller 1952: 155 (dtm), Rad. 1952: 267, Hammen 1956 (dm), Wom. & A. 1957 (xl).

Records. NEW GUINEA. Rodents—*Rattus conatus, R. exulans concolor, R. ruber ringens.* Primate—Man. Also from unrecorded hosts. (See also *S. blestowei*, ? syn.)

Schongastia vieta Gater

(1) **Schongastia vieta vieta** Gater

Schongastia (*Schongastia*) *vieta*, Wom. 1952: 163 (35) d, 387 (105) DN.

Schongastia vieta, Gun. 1952: 24, W. & F. 1952: 89, Audy *et al.* 1953: 34 (c), Rad. 1954: 267, Audy 1956*d*: 95 (ct).

Schongastia vieta vieta, Audy 1954*b*: 151 (t), Wom. & A. 1957 (xl).

Records. BURMA. Rodents—*Bandicota bengalensis, Rattus r. khyensis.*

(2) **Schongastia vieta maldiviensis** Radford

Schongastia (*Schongastia*) *maldiviensis*, Wom. 1952: 169 (38) d, 381 (102) DN.

Schongastia maldiviensis, Gun. 1952: 25, Rad. 1954: 267.

Schongastia vieta, in part, W. & F. 1952: 89 (s).

Schongastia vieta maldiviensis, Audy 1954*b*: 151 (t), Wom. & A. 1957 (xl).

Schongastia (?) westraliensis* (Womersley)

Schongastia westraliensis (=*Microtrombidium westraliense* Wom., 1934, not *Schongastia westraliense* Wom. 1934=*S. newmani, nom.nov.*, Wom. 1952: 219 (d), 427 (x)), Wom. 1952: 383 (104) dA.

Schongastia whartoni Womersley

Schongastia (*Schongastia*) *whartoni* Wom., 1952: 161 (34) D.
Records. NEW GUINEA. Unattached.

Genus **Oenoschongastia** Womersley & Kohls, 1947

Type: *Oenoschongastia cana* Womersley & Kohls, 1947

Oenoschongastia cana Womersley & Kohls

Oenoschongastia cana, Wom. 1952: 243 (66) d, W. & F. 1952: 87, Gun. 1952: 36, Audy 1954*b*: 151 (t), Rad. 1954: 267, Wom. & A. 1957 (xl).

Genus **Radfordiana** Womersley, 1952

Type: *Radfordiana rostrata* Womersley, 1952

Radfordiana rostrata Womersley

Radfordiana rostrata Wom., 1952: 242 (66) D, Audy 1954*b*: 151 (t), Rad. 1954: 267, Wom. & A. 1957 (l).
Records. NEW GUINEA. Unattached. Bird (?)—Nest of *Megapodius*. Reptile—'Armed lizard'.

Genus **Euschongastia** Ewing, 1938, *sensu lato*

Type: (*Euschongastia americana* Ewing, synonym)=*Schongastia sciuricola* Ewing, 1925

Subgenus **Derrickiella**† Audy & Domrow, 1957

Type: *Neoschongastia smithi* Womersley, 1939

Euschongastia (Derrickiella) cairnsensis (Womersley & Heaslip)

Schongastia (*Ascoschongastia*) *cairnsensis*, Wom. 1952: 226 (65) d.
Schongastia (*Ascoschongastia*) *cairnsensis* var. *gateri*, Wom. 1952: 227 (65) d.
Neoschongastia cairnsensis, Gun. 1952: 31.

* The proper genus is unknown but may be *Euschongastia*.

† This subgenus includes the *similis*-group and part of the *lacunosa* group (not subgenus *Walchiella*) of Audy 1954*b*: 154, 155. The subgenus is divided by its authors into a typical *smithi*-group, and a *similis*-group (new sense) comprising the species *dasycerci, derricki, hirsti, shieldsi* and *similis*.

Euschongastia cairnsensis, W. & F. 1952: 74, Audy 1954*b*: 155, Rad. 1954: 264, Dom. 1956*a*: 203 (c).

Euschongastia cairnsensis var. *gateri*, Rad. 1954: 264.

Euschongastia (*Derrickiella*) *cairnsensis*, Audy & Dom. 1957 (tx), Wom. & A. 1957 (xl).

Euschongastia (Derrickiella) dasycerci (Womersley)

Schongastia (*Ascoschongastia*) *dasycerci*, Wom. 1952: 216 (54) d.

Neoschongastia dasycerci, Gun. 1952: 28.

Euschongastia dasycerci, W. & F. 1952: 75, Audy 1954*b*: 155 (t), Rad. 1954: 264.

Euschongastia (*Derrickiella*) *dasycerci*, Audy & Dom. 1957 (tx), Wom. & A. 1957 (xl).

Records. AUSTRALIA. Rodent—*Rattus greyi murrayi*.

Euschongastia (Derrickiella) derricki (Womersley)

Schongastia (*Ascoschongastia*) *derricki*, Wom. 1952: 215 (58, 59) d.

Euschongastia derricki, W. & F. 1952: 76, Audy 1954*b*: 155 (t), Rad. 1954: 264, Dom. 1956*a*: 203 (c).

Neoschongastia derricki, Gun. 1952: 30.

Euschongastia (*Derrickiella*) *derricki*, Audy & Dom. 1957 (dt), Wom. & A. 1957 (xl).

Euschongastia (Derrickiella) heaslipi (Womersley & Heaslip)

Schongastia (*Ascoschongastia*) *heaslipi*, Wom. 1952: 221 (62) d.

Neoschongastia heaslipi, Gun. 1952: 32.

Euschongastia heaslipi, W. & F. 1952: 77, Rad. 1954: 265.

Euschongastia (*Walchiella*)(!) *heaslipi*, Audy 1954*b*: 153 (t), wrong subgenus.

Euschongastia (*Derrickiella*) *heaslipi*, Audy & Dom. 1957 (tx), Wom. & A. 1957 (xl).

Euschongastia (Derrickiella) hirsti (Womersley & Heaslip)

Schongastia (*Ascoschongastia*) *hirsti*, Wom. 1952: 218 (60) d.

Neoschongastia hirsti, Gun. 1952: 32.

Euschongastia hirsti, W. & F. 1952: 77, Audy 1954*b*: 155, Rad. 1954: 265, Dom. 1956*a*: 204.

Euschongastia (*Derrickiella*) *hirsti*, Audy & Dom. 1957 (tx), Wom. & A. 1957 (xl).

Records. AUSTRALIA. Rodent—'Rat'.

Euschongastia (Derrickiella) lawrencei (Womersley)

Schongastia (*Ascoschongastia*) *lawrencei* Wom., 1952: 223 (63) d, *nom.nov.* for *S.* (*A.*) *guntheri* (Wom. & Heaslip, 1943), preoccupied by *S.* (*A.*) *guntheri* (Radford 1942).

Neoschongastia (*guntheri*) (*sic*!), Gun. 1952: 31.

Euschongastia lawrencei, W. & F. 1952: 78, Audy 1954*b*: 154 (tx), Rad. 1954: 265.

Euschongastia (*Derrickiella*) *lawrencei*, Audy & Dom. 1957 (tx), Wom. & A. 1957 (xl).

Euschongastia (Derrickiella) newmani (Womersley)

Schongastia (*Ascoschongastia*) *newmani* Wom. 1952: 427 (*nom.nov.*) for *S.* (*A.*) *westraliense* (Wom. 1934: 215), Wom. 1952: 219.

Schongastia (*Ascoschongastia*) *westraliensis*, Wom. 1934: 215, 1952: 219 (61) d (preoccupied by *S. westraliensis* (Wom. 1934: 188), Wom. 1952: 383 (104) dA, *q.v.*).

Neoschongastia westraliense, Gun. 1952: 29.

Euschongastia westraliensis, W. & F. 1952: 82.

Euschongastia westralensis (!), Rad. 1954: 266.

Euschongastia (*Derrickiella*) *newmani*, Audy & Dom. 1957 (tx), Wom. & A. 1957 (xl).

Euschongastia (Derrickiella) perameles (Womersley)

Schongastia (*Ascoschongastia*) *perameles*, Wom. 1952: 213 (56) d.

Neoschongastia perameles, Gun. 1952: 30.

Euschongastia perameles, W. & F. 1952: 80, Audy 1954*b*: 155 (t), Rad. 1954: 265, Domrow 1955 (DN), 1956*a*: 204 (dc).

Euschongastia (? *Derrickiella*) *perameles*, Audy & Dom. 1957 (dctx), Wom. & A. 1957 (xl).

Records. AUSTRALIA. Marsupial—*Perameles nasuta*.

Euschongastia (Derrickiella) peregrina (Womersley)

Schongastia (*Ascoschongastia*) *peregrina* Wom., 1952: 220 (61) d.

Euschongastia peregrina, Rad. 1954: 265.

Euschongastia (*Derrickiella*) *peregrina*, Audy & Dom. 1957 (xl), Wom. & A. 1957 (xl).

Records. AUSTRALIA. Unattached.

Euschongastia (Derrickiella) phascogale (Womersley & Heaslip)

Schongastia (*Ascoschongastia*) *phascogale*, Wom. 1952: 219 (60) d.

Neoschongastia phascogale, Gun. 1952: 32.

Euschongastia phascogale, W. & F. 1952: 80, Rad. 1954: 266, Dom. 1956*a*: 204 (c).

Euschongastia (*Derrickiella*) *phascogale*, Audy & Dom. 1957 (lx), Wom. & A. 1957 (xl).

Records. AUSTRALIA. Marsupial—*Thylacis obesulus*.

Euschongastia (Derrickiella) shieldsi (Gunther)

Schongastia (*Ascoschongastia*) *trichosuri*, Wom. 1952: 217 (ds) in part.

Neoschongastia shieldsi, Gun. 1952: 31.

Euschongastia shieldsi, Rad. 1954: 266.

Euschongastia ? *shieldsi*, W. & F. 1952: 81.

Euschongastia (*Derrickiella*) *shieldsi*, Audy & Dom. 1957 (tx), Wom. & A. 1957 (xl).

Euschongastia (Derrickiella) similis (Womersley & Heaslip)

Schongastia (*Ascoschongastia*) *similis*, Wom. 1952: 214 (58) d.

Neoschongastia similis, Gun. 1952: 32.

Euschongastia similis, W. & F. 1952: 81, Rad. 1954: 266.

Euschongastia (*Derrickiella*) *similis*, Audy & Dom. 1957 (tx), Wom. & A. 1957 (xl).

Euschongastia (Derrickiella) smithi (Womersley)

Schongastia (*Ascoschongastia*) *smithi*, Wom. 1952: 225 (65) d.

Neoschongastia smithi, Gun. 1952: 31.

Euschongastia smithi, W. & F. 1952: 81, Rad. 1954: 266, Domrow 1955 (DN), 1956*a* (c).

Euschongastia (*lacunosa*-group) *smithi*, Audy 1954*b*: 154 (t), wrong group.

Euschongastia (*Derrickiella*) *smithi*, Audy & Dom. 1957 (dtx), Wom. & A. 1957 (xl).

Euschongastia (Derrickiella) trichosuri (Womersley)

Schongastia (*Ascoschongastia*) *trichosuri*, Wom. 1952: 217 (59) dx.

Neoschongastia trichosuri, Gun. 1952: 31.

Euschongastia trichosuri, W. & F. 1952: 82, Audy 1954*b*: 155, Rad. 1954: 266, Dom. 1956*a*.

Euschongastia (*Derrickiella*) *trichosuri*, Audy & Dom. 1957 (tx), Wom. & A. 1951 (xl).

Euschongastia (Derrickiella) wongabelensis (Womersley)

Schongastia (*Ascoschongastia*) *wongabelensis* Wom., 1952: 224 (63) D.

Euschongastia (? *lacunosa*-group) *wongabelensis*, Audy 1954*b*: 155 (t), wrong group.

Euschongastia wongabelensis, Rad. 1954: 266, Dom. 1956*a*: 204 (c).

Euschongastia (*Derrickiella*) *wongabelensis*, Audy & Dom. 1957 (tx), Wom. & A. 1957 (xl).

Records. AUSTRALIA. Rodent—'Water rat'. *Rattus assimilis*. Also unidentified host.

Subgenus **Trombewingia** Fonseca, 1955

Type: *Euschongastia* (*Trombewingia*) *bakeri* Fonseca, 1955

Euschongastia (Trombewingia) foliata (Gunther)

Schongastia (*Ascoschongastia*) *foliata*, Wom. 1952: 173 (40) d.

Euschongastia foliata, W. & F. 1952: 76, Audy 1954*b*: 155 (t), Rad. 1954: 155.

Neoschongastia foliata, Gun. 1952: 31.

Euschongastia (*Trombewingia*) *foliata* Wom. & A. 1957 (xl.)

Euschongastia (Trombewingia) mccullochi (Womersley)

Schongastia (*Ascoschongastia*) *mccullochi*, Wom. 1952: 172 (39) d.
Neoschongastia mccullochi, Gun. 1952: 32.
Euschongastia mccullochi, W. & F. 1952: 79, Audy 1954*b*: 155 (t), Rad. 1954: 265.
Euschongastia (*Trombewingia*) *mccullochi*, Wom. & A. 1957 (xl).

Euschongastia (Trombewingia) uromys (Womersley & Kohls)

Schongastia (*Ascoschongastia*) *uromys*, Wom. 1952: 172 (40) d.
Neoschongastia uromys, Gun. 1952: 35.
Euschongastia uromys, W. & F. 1952: 82, Audy 1954*b*: 155 (t), Rad. 1954: 266.
Euschongastia (*Trombewingia*) *uromys*, Wom. & A. 1957 (xl).

Subgenus **Walchiella*** Fuller, 1952

Type: *Trombicula oudemansi* Walch, 1922

Euschongastia (Walchiella) asonluca Traub & Audy

Euschongastia (*Walchiella*) *asonluca* Traub & A., 1954*b*: 84 (87) D, Audy 1954*b*: 153, 1956*d*: 96 (cd), Audy & Dom. 1957 (t).
Records. BORNEO. Insectivore—*Tupaia tana*. MALAYA. Insectivore—*Hylomys suillus*.

Euschongastia (Walchiella) calunosa Traub & Audy

Euschongastia (*Walchiella*) *calunosa* Traub & A., 1954*b*: 84 (85) D, Audy 1954*b*: 153, Audy & Dom. 1957 (dt).
Records. BORNEO. Rodent—*Rattus r. baluensis*.

Euschongastia (Walchiella) impar (Gunther)

Schongastia (*Schongastia*) *oudemansi*, in part, Wom. 1952: 153 (32) dt, ? 380 (101) DN.
Neoschongastia impar, Gun. 1952: 29.
Walchiella oudemansi, in part, Fuller 1952: 221 (s), W. & F. 1952: 95 (t).
Euschongastia (*Walchiella*) *impar*, Audy 1954*b*: 153 (tx), Audy & Dom. 1957 (dtx), Wom. & A. 1957 (xl).
Schongastia impar, Rad. 1954: 266.

Euschongastia (Walchiella) lacunosa (Gater)

Schongastia (*Ascoschongastia*) *lacunosa*, Wom. 1952: 197 (51) d, 387 (114) DN (nymph mis-identified), IMR 1952: 50 (bc).
Neoschongastia lacunosa, Gun. 1952: 28.

* Fuller, and Wharton & Fuller, place this in the subfamily Gahrliepiinae (Walchiinae) but this is no longer acceptable. Radford omits *Walchiella* from his list. *Walchiella* includes the *lacunosa*-group of Audy (1954*b*).

Euschongastia lacunosa, W. & F. 1952: 78, Audy 1954*b*: 154 (t), 1956*d*: 97 (cd), Rad. 1954: 265.

Euschongastia (*Walchiella*) *lacunosa*, Audy & Dom. 1957 (dtx), Wom. & A. 1957 (xl), Har. 1957*a* (c), 1957*b* (b).

Euschongastia (Walchiella) lewthwaitei (Womersley)

Schongastia (*Schongastia*) *lewthwaitei* Wom., 1952: 154 (111) D.

Schongastia (*Ascoschongastia*) *lacunosa*, in part, Wom. 1952: 198, *err. det.*

Euschongastia (*Walchiella*) *lewthwaitei*, Audy 1954*b*: 153 (t), Audy & Dom. 1957 (dt), Wom. & A. 1957 (xl).

Records. INDIA, North-east. Rodent—*Tupaia glis belangeri*. Insectivore—*Hadromys humei*.

Euschongastia (Walchiella) nadchatrami (Womersley)

Schongastia (*Ascoschongastia*) *nadchatrami* Wom., 1952: 200 (111) D (*not* 387 (114); nymph mis-identified).

Euschongastia nadchatrami, Audy 1954*b*: 154 (t), 1956*d*: 97 (cd), Rad. 1954: 265.

Euschongastia (*Walchiella*) *nadchatrami*, Audy & Dom. 1957 (dtx), Wom. & A. 1957 (xl), Har. 1957*a* (c), 1957*b* (b).

Records. MALAYA. Rodents—*Rattus rajah*, *R. sabanus*.

Euschongastia (Walchiella) oudemansi (Walch)

Schongastia (*Schongastia*) *oudemansi*, Wom. 1952: 152 (32) d (not fig. 101 K (N)).

Schongastia oudemansi, Gun. 1952: 23, IMR 1952: 50 (bc), Rad. 1954: 267.

Walchiella oudemansi, Fuller 1952: 221 (tdx), W. & F. 1952: 95.

Euschongastia (*Walchiella*) *oudemansi*, Audy 1954*b*: 126, 153 (t), 1956*d*: 96 (cd), Audy & Dom. 1957 (dtx), Wom. & A. 1957 (xl), Har. 1957 (b).

Euschongastia (Walchiella) sarawakensis (Womersley)

Schongastia (*Ascoschongastia*) *sarawakensis* Wom., 1952: 201 (111) D.

Euschongastia sarawakensis, Audy 1954*b*: 154 (t), Rad. 1954: 266.

Euschongastia (*Walchiella*) *sarawakensis*, Audy & Dom. 1957 (tx), Wom. & A. 1957 (xl).

Records. BORNEO. Insectivore—*Echinosorex gymnurus*. (Radford, 1954: 266, wrongly records *Sorex minutus gymnurus*, which is a true shrew from Greece.)

Euschongastia (Walchiella) traubi (Womersley)

Schongastia (*Ascoschongastia*) *traubi* Wom., 1952: 222 (62) D.

Euschongastia (*Walchiella*) *traubi*, Audy 1954*b*: 153 (tx), 1956*d*: 96 (cd), Audy & Dom. 1957 (dtx), Wom. & A. 1957 (xl).

Euschongastia traubi, Rad. 1954: 266.

Records. BURMA, North. Insectivore—*Suncus* sp. MALAYA (requires confirmation). Rodent—*Rattus sabanus*.

Subgenus uncertain*

Euschongastia andromeda Womersley

Euschongastia andromeda Wom., 1954: 80 (d), Audy & Dom. 1957 (dt).
Records. AUSTRALIA. Unattached.

Euschongastia antipodianum (Hirst)

Schongastia (*Ascoschongastia*) *antipodianum*, Wom. 1952: 188 (47) D.
Neoschongastia antipodianum, Gun. 1952: 28.
Euschongastia antipodianum, W. & F. 1952: 73, Audy 1954*b*: 155, Rad. 1954: 264, Dom. 1956*a*: 203 (c), Audy & Dom. 1957 (dt), Wom. & A. 1957 (xl).
Records. AUSTRALIA. Marsupial—*Antechinus flavipes.*

Euschongastia cassiope (Womersley)

Schongastia (*Ascoschongastia*) *cassiope* Wom., 1952: 187 (47, 48) D.
Euschongastia cassiope, Audy 1954*b*: 155 (t), Rad. 1954: 264, Wom. & A. 1957 (xl).
Euschongastia lappacea (*q.v.*), ? synonym, Audy & Dom. 1957 (t).
Records. AUSTRALIA. Unattached.

Euschongastia coorongense (Hirst)

Schongastia (*Ascoschongastia*) *coorongense*, Wom. 1952: 192 (50) d.
Neoschongastia coorongense, Gun. 1952: 28.
Euschongastia coorongense, W. & F. 1952: 75, Audy 1954*b*: 155, Rad. 1954: 264, Wom. & A. 1957 (xl).
Records. AUSTRALIA. Rodent—*Rattus greyi* ? *murrayi.*

Euschongastia crinita (Womersley)

Schongastia (*Ascoschongastia*) *crinita* Wom., 1952: 185 (46) D.
Euschongastia crinita, Audy 1954*b*: 155 (t), Rad. 1954: 264, Audy & Dom. 1956 (dt), Wom. & A. 1957 (xl).
Records. NEW GUINEA. Marsupial—*Echymipera kalubu kalubu.*

Euschongastia dumosa (Womersley)

Schongastia (*Ascoschongastia*) *dumosa* Wom., 1952: 189 (48) D.
Euschongastia dumosa, Rad. 1954: 264, Wom. & A. 1957 (xl).
Euschongastia procana Wom., 1954: 77 (D), syn., Audy & Dom. 1957 (dtx).
Records. AUSTRALIA. Unattached.

* Audy & Domrow, in a revision of the Oriental and Australasian species at present accommodated in *Euschongastia*, recognize the following species-groups, a few of which are monotypic: (*a*) *andromeda, parva, popei*, (*b*) *dumosa, coorongensis, popei*; (*c*) *lappacea* = *cassiope* syn. ?, *echymipera, innisfailensis, queenslandica*; (*d*) *mohri, crinita,* ? *petrogale*; (*e*) *mackerrasae*; (*f*) *schlugeri*. Species *antipodianum* and *perameles* were left unallocated though the latter is probably a *Derrickiella*. Species *E.* (?) *oculicola*, which is closer to *Ascoschongastia*, was left unallocated (see Audy & Wom. 1957).

Euschongastia echymipera (Womersley & Kohls)

Schongastia (*Ascoschongastia*) *echymipera*, Wom. 1952: 193 (50) d.

Neoschongastia echymipera, Gun. 1952: 35.

Euschongastia echymipera, W. & F. 1952: 76, Audy 1954*b*: 155, Rad. 1954: 265, Wom. & A. 1957 (xl).

Euschongastia innisfailensis (Womersley & Heaslip)

Schongastia (*Ascoschongastia*) *innisfailensis*, Wom. 1952: 194 (51) d.

Neoschongastia innisfailensis Gun. 1952: 32.

Neoschongastia melomys, Gun. 1952: 32. (Note: *N. melomys* = *E. innisfailensis* and *E. lappacea*, *q.v.*)

Neoschongastia bushlandi, Gun. 1952: 35.

Euschongastia melomys, W. & F. 1952: 79, Rad. 1954: 265.

Euschongastia bushlandi, W. & F. 1952: 74, Rad. 1954: 264.

Euschongastia innisfailensis, W. & F. 1952: 77, Audy 1954*b*: 155 (t), Rad. 1954: 265, Audy & Dom. 1957 (dt), Wom. & A. 1957 (xl).

Records. AUSTRALIA. Unattached. Rodent—*Melomys littoralis* (also the original record for *N. melomys*, syn.). NEW GUINEA. Bird—*Megapodius* sp. (record for *bushlandi*, syn.).

Euschongastia lappacea (Womersley) ? synonym

Schongastia (*Ascoschongastia*) *lappacea* Wom., 1952: 190 (49) D.

Neoschongastia melomys Wom. & Heaslip, 1953: 110, Gun. 1952: 32, in part.

Euschongastia melomys, Audy 1954*b*: 155, Rad. 1954: 265, in part.

Euschongastia lappacea, Audy 1954*b*: 155 (t), Rad. 1954: 265, Wom. & A. 1957 (xl).

Euschongastia cassiope (*q.v.*), ? synonymous with, Audy & Dom. 1957 (t).

Records. AUSTRALIA. Unattached. Rodents—'Rats' (series mis-identified as *E. melomys* syn. *E. innisfailensis*). Marsupials—*Wallabia agilis*.

Euschongastia mackerrasae (Womersley)

Schongastia (*Ascoschongastia*) *mackerrasae* Wom., 1952: 182 (45) D.

Euschongastia mackerrasae, Audy 1954: 155 (t), Rad. 1954: 265, Audy & Dom. 1957 (dt), Wom. & A. 1957 (xl).

Records. AUSTRALIA. Unattached.

Euschongastia mohri (Womersley)

Schongastia (*Ascoschongastia*) *mohri* Wom., 1952: 184 (46) D.

Euschongastia mohri, Audy 1954: 155 (t), Rad. 1954: 265, Wom. & A. 1957 (xl).

Records. NEW GUINEA. Rodent—*Rattus exulans concolor*. Marsupial—*Echymipera kalubu kalubu*.

Euschongastia (?) oculicola (Womersley)

Schongastia (*Schongastia*) *oculicola* Wom., 1952: 167 (37) D, 383 (103) DN.
? *Doloisia* (*oculicola*-group) *oculicola*, Audy 1954*b*: 159 (tx).
Schongastia oculicola, Rad. 1954: 267.
Euschongastia (*oculicola*-group, *Laurentella* complex) *oculicola*, Audy 1956*a*: 20 (dt).
Euschongastia (?) *oculicola*, Audy & Wom. 1957 (dt), Wom. & A. 1957 (xl).
Records. CEYLON. Rodent—*Mus cervicolor fulvidiventris*.

Euschongastia parva Womersley

Euschongastia parva Wom., 1954: 75 (D), Audy & Dom. 1957 (dt).
Records. AUSTRALIA. Unattached.

Euschongastia petrogale (Womersley)

Schongastia (*Ascoschongastia*) *petrogale*, Wom. 1952: 184 (45) d.
Neoschongastia petrogale, Gun. 1952: 29.
Euschongastia petrogale, W. & F. 1952: 80, Rad. 1954: 265, Audy & Dom. 1957 (dt), Wom. & A. 1957 (xl).

Euschongastia philippensis (Philip & Woodward)

Schongastia (*Ascoschongastia*) *philippensis*, Wom. 1952: 181 (45) d.
Neoschongastia philippensis, Gun. 1952: 33.
Euschongastia philippensis, W. & F. 1954: 80, Audy 1954*b*: 155, Rad. 1954: 266, Audy & Dom. 1957 (dt), Wom. & A. 1957 (xl).
Records. PHILIPPINES. ? Rodents—? Rats. AUSTRALIA. Marsupial—*Onychogalea unguifer*.

Euschongastia popei Womersley

Euschongastia popei, Wom., 1952: 76 (D).
Records. AUSTRALIA. Rodent—*Rattus fuscipes assimilis*.

? Euschongastia [Trombiculindus] pseudomys* (Womersley)

Schongastia (*Ascoschongastia*) *pseudomys* Wom., 1952: 171 (39) D.
Euschongastia (*foliata*-group)(!) *pseudomys*, Audy 1954*b*: 155 (t), wrong group.
Euschongastia pseudomys, Rad. 1954: 266.
? *Euschongastia* (? *Trombiculindus*) *pseudomys*, Wom. & A. 1957 (dtxl), Audy & Dom. 1957 (t).
Records. AUSTRALIA. Marsupial—*Trichosurus vulpecula ? johnstoni*.

* This is not a *Trombewingia* but appears to belong to *Trombicula*, subgenus *Trombiculindus*. In the absence of sensillae it is left where it is pending further studies. The name *pseudomys* is a misnomer owing to a faulty host-identification.

Euschongastia queenslandica (Womersley)

Schongastia (*Ascoschongastia*) *queenslandica*, Wom. 1952: 190 (48) d.
Neoschongastia queenslandica, Gun. 1952: 31.
Euschongastia queenslandica, W. & F. 1952: 81, Audy 1954*b*: 155 (t), Rad. 1954: 266, Wom. & A. 1957 (xl).

Euschongastia schlugeri Radford

Euschongastia schlugeri Rad., 1953*c*: 210 (D), Audy & Dom. 1957 (t).
Records. INDIA, Peninsular. Rodent—Rat ['*Rattus* sp.' (?)].

Euschongastia womersleyi (Gunther)

Schongastia (*Ascoschongastia*) *womersleyi*, Wom. 1952: 191 (49) d.
Neoschongastia womersleyi, Gun. 1952: 31.
Euschongastia womersleyi, W. & F. 1952: 82, Audy 1954*b*: 155 (t), Rad. 1954: 266, Wom. & A. 1957 (xl).

Genus **Ascoschongastia*** Ewing, 1946

Type: *Neoschongastia malayensis* Gater, 1932

Subgenus **Ascoschongastia** Ewing, 1946

Type: *Neoschongastia malayensis* Gater, 1932

Ascoschongastia (Ascoschongastia) capillata Radford

Ascoschongastia capillata Rad., 1953*a*: 235 (D), 1954: 264.
Records. INDIA, Peninsular. Rodent—'Rat (*Rattus* sp.)' (?).

Ascoschongastia (Ascoschongastia) malayensis (Gater)

Schongastia (*Ascoschongastia*) *malayensis*, Wom. 1952: 199 (51) d, 384 (104) DN.
Ascoschongastia malayensis, Gun. 1952: 36, W. & F. 1952: 71, Audy 1954*b*: 155 (t), 1956*a*: 19 (btc), 1956*d*: 91, 97 (cd), Rad. 1954: 264.
Euschongastia malayensis, Rad. 1954: 265.
Ascoschongastia (*Ascoschongastia*) *malayensis*, Dom. 1957*b* (dNt), Wom. & A. 1957 (xl), Harrison 1957 (b).

* This genus was originally raised to accommodate those species which were neither *Euschongastia s.str.* (Old World) nor *Neoschongastia s.str.* (distinctive bird-chiggers); but an unfortunate choice of the type species has led to confusion. '*Ascoschongastia sensu lato*', without qualification, now has no clear meaning; it was used as a very broad and heterogeneous subgenus by Womersley (1952), and is accepted here in a different sense, but also broadly, after Domrow (1957*b*). *Ascoschongastia s.str.* is clearly a slightly modified offshoot of the *Laurentella* stem, which itself arises, apparently, from a common stem with the *Trombicula panieri* group (represented in the Oriental region by species such as *munda*).

Ascoschongastia (?) masta Traub & Sundermeyer

Schongastia (*Ascoschongastia*) *masta*, Wom. 1952: 183 (68) d.
Ascoschongastia masta, W. & F. 1952: 71, Audy 1954*b*: 155, Rad. 1954: 264.
Euschongastia masta, Rad. 1954: 265.
Ascoschongastia (?) *masta*, Audy & Wom.1957 (dt), Wom. & A. 1957 (xl).

Subgenus **Laurentella** Audy, 1956
Type: *Schongastia indica* Hirst, 1915

Ascoschongastia (Laurentella) audyi (Womersley)

Schongastia (*Ascoschongastia*) *audyi* Wom., 1952: 205 (54, 57) D, 386 (105) DN, 2a (x).
Euschongastia indica, Traub *et al.* 1950 (m) (identity amended in Traub & A. 1954*b*: 77).
Euschongastia species 'S', Audy & Har. 1951: 390 (c).
Euschongastia audyi, IMR 1952: 50 (bc), 93 (c), Audy *et al.* 1953 (c), Traub & A. 1954*b*: 77 (m), 81 (t), Audy 1954*b*: 154 (t), Har. 1954: 181 (b), 1956 (c), Rad. 1954: 264.
Euschongastia (*Laurentella*) *audyi*, Audy 1956*a*: 10, 21 (bctx), 1956*b*: 64 (c), 1954*d*: 96 (cd), Wom. & A. 1957 (lx).
Ascoschongastia (*Laurentella*) *audyi*, Dom. 1957*b* (dNtx), Har. 1957*a* (c), 1957*b* (b).
Records. SOUTH BURMA. Rodents—*Rattus r. khyensis.* BORNEO. Rodents—Commonest on *Callosciurus notatus, C. tenuis, C. prevosti*; also *C. adamsi, C. lowii, Dremomys everetti, Nannosciurus whiteheadi, Rattus argentiventer, R. mulleri, R. whiteheadi.* Insectivores—*Tupaia minor, T. tana.* THAILAND. Rodents—*Rattus r. thai, R. rattus* subsp., *Callosciurus* sp., *C. erthraeus, C. notatus miniatus.* MALAYA. Rodents—Dominant on *Callosciurus nigrovittatus, C. notatus, C. tenuis*; also *C. caniceps, Rattus canus, R. jalorensis*; casual on *R. alticola, R. annandalei, R. argentiventer, R. rajah, R. r. diardi, R. sabanus, C. lowii, Lariscus insignis, Rhinosciurus laticaudatus.* Insectivores—*Cynocephalus variegatus, Hylomys suillus, Tupaia glis, T. minor.*

Ascoschongastia (Laurentella) daria Traub & Audy

Euschongastia daria Traub & A. 1954: 81 (D), IMR 1952: 93 (1) (no. 46).
Euschongastia (*Laurentella*) *daria*, Audy 1956*a*: 11 (tx), 1956*b*: 64 (c).
Ascoschongastia (*Laurentella*) *daria*, Dom. 1957*b* (tx).
Records. BORNEO. Rodents—*Nannosciurus whiteheadi.* Insectivores—*Tupaia montana baluensis, T. m. minor.* Bird—Chick of ground-bird.

Ascoschongastia (Laurentella) debilis* (Gater)

Schongastia (*Ascoschongastia*) *debilis*, Wom. 1952: 203 (53) d.
Neoschongastia (?) *debilis*, Gun. 1952: 28.

* The *debilis*-group, comprising *debilis, labuanensis*, and two undescribed species, is distinct from the typical *indica*-group and from a third group (*TAA*-group, Audy 1956*a*: 15 (8)), which is exclusively intranasal.

Euschongastia debilis, W. & F. 1952: 75, Audy 1954*b*: 154 (t), Rad. 1954: 264.
Euschongastia (*Laurentella*) *debilis*, Audy 1956*a*: 14 (8) (dtc), 1956*d*: 96 (cd).
Ascoschongastia (*Laurentella*) *debilis*, Dom. 1957 (tx), Wom. & A. 1957 (xl).
Records. MALAYA. Rodents—*Rattus annandalei*, *R. jalorensis*, *R. mulleri*, *R. sabanus*, *R. whiteheadi* (all casual). Insectivores—*Crocidura malayana*, *Chimmarogale* (water-shrew, new species).

Ascoschongastia (Laurentella) indica (Hirst)

Trombicula muris, Fletcher *et al.* 1928 (dc), Hayakawa & Hokari 1946, syn. of *E.* (*L.*) *indica*, homonym of *T.* (*Neotrombicula*) *muris* (Ouds.).
Trombicula indica (*muris*) (*sic*!), Patton & Evans 1929: 655, Mehta 1937: 354.
Euschongastia sp. nr. *indica*, Audy 1950: 193 (c), 1956*b* (cx).
Schongastia (*Ascoschongastia*) *indica*, Audy 1952: 208, 378 (100) dN, 2c (x).
Neoschongastia indica, Gun. 1952: 26.
Euschongastia (*indica*-group) *indica*, Audy 1954: 154 (t).
Euschongastia indica, IMR 1950: 64 (c), 1952: 93 (c), 1953: 65, W. & F. 1952: 77, Audy 1954*b*: 193 (t), Audy & Har. 1954: 17 (c), Audy *et al.* 1953 (bc), Traub & A. 1954*b*: 77 (tmx), Traub & E. 1954: 103, Harrison 1954: 181 (b), 1956 (c), Rad. 1954: 264, Dom. 1955*b*: 132 (tN).
Euschongastia cockingsi, W. & F. 1952: 75, Rad. 1954: 264.
Euschongastia muris, Rad. 1954: 265.
Ascoschongastia monteli Andre, 1954 (D), Le Gac *et al.* 1954 (d), Audy 1956*a*: 10 (s).
Euschongastia (*Laurentella*) *indica*, Audy 1956*a*: 7, 21 (bctx), 1956*b*: 64 (c), 1956*d*: 96 (cd).
Ascoschongastia (*Laurentella*) *indica*, Dom. 1957*b* (dNtx), Wom. & A. 1957 (xl), Har. 1957*a* (c), 1957*b* (b).
Records. INDIA, North-east. Rodents—*Rattus r. bullocki*. Insectivore—*Tupaia glis* ? *belangeri*. Carnivore—*Herpestes urva*. INDIA, Peninsular. Rodents—'House rats' (from widespread towns), *Tatera indica*. Insectivore—*Suncus* ? *murinus*. INDIAN OCEAN, Car Nicobar. Rodent—*Rattus rattus* subsp. HONG KONG. Rodent—*R. norvegicus* (in houses), major hosts; *Callosciurus tenuis*, *R. exulans*, *R. cremoriventer*. Casually on *C. caniceps*, *C. lowii*, *C. nigrovittatus*, *C. notatus*, *R. annandalei*, *R. argentiventer*, *R. bowersi*, *R. canus*, *R. mulleri*, *R. sabanus*. Insectivores—*Tupaia glis*, *T. minor*. Carnivore—*Paradoxurus hermaphroditus*. Primate—*Nyctecebus coucang*. Arthropod—two stragglers on *Sphaeropaeus globus magicus* (Diplopoda Oniscomorpha). BORNEO. Rodents—*Rattus jalorensis*, *R. r. diardi*; *C. notatus*, *C. prevosti*, *R. whiteheadi*. THAILAND. Rodents—*Bandicota indica*, *C. nigrovittatus*, 'grey squirrel', *Menetes berdmorei*. Insectivores—'Shrew', *Tupaia* sp. Carnivore—'Civet', 'Mongoose'.

Ascoschongastia (Laurentella) indicella Traub & Audy

Euschongastia indicella Traub & Audy, 1954: 77 (D), IMR 1952: 93 (no. 47), Audy 1954*b*: 154 (t).

Euschongastia (*Laurentella*) *indicella*, Audy 1956*a*: 12 (tcx).

Ascoschongastia (*Laurentella*) *indicella*, Dom. 1957*b* (tx).

Records. BORNEO. Rodent—*Chiropodomys legatus*.

Ascoschongastia (Laurentella) labuanensis (Womersley)

Schongastia (*Ascoschongastia*) *labuanensis* Wom. 1952: 204 (53) D, 2c (tx).

Euschongastia labuanensis, Traub & A. 1954: 77 (t), Audy 1954*b*: 154 (t), Rad. 1954: 265.

Euschongastia (*Laurentella*) *labuanensis*, Audy 1956*a*: 14 (tcx), Wom. & A. 1957 (xl).

Ascoschongastia (? *Laurentella*) *labuanensis*, Dom. 1957*b* (tx).

Records. BORNEO. Rodent—'Rat'.

Ascoschongastia (Laurentella) lorius (Gunther)

Schongastia (*Ascoschongastia*) *lorius*, Wom. 1952: 207 (55, 57) d.

Euschongastia lorius, W. & F. 1952: 78, Traub & A. 1954: 79 (t), Audy 1954*b*: 154 (t), Rad. 1954: 265.

Neoschongastia lorius, Gun. 1952: 30.

Euschongastia (*Laurentella*) *lorius*, Audy 1956*a*: 12 (tcx), Wom. & A. 1957 (xl).

Ascoschongastia (*Laurentella*) *lorius*, Dom. 1957*b* (tx).

Ascoschongastia (Laurentella) ocellifera Traub & Audy

Euschongastia ocellifera, Traub & Audy 1954: 79 (D), IMR 1952: 93 (no. 49).

Euschongastia (*Laurentella*) *ocellifera*, Audy 1956*a*: 12 (tx).

Ascoschongastia (*Laurentella*) *ocellifera*, Dom. 1957*b* (tx), Wom. & A. 1957 (xl).

Records. BORNEO. Rodents—*Chiropodomys legatus*.

Ascoschongastia (Laurentella) rattus (Womersley & Heaslip)

Schongastia (*Ascoschongastia*) *rattus*, Wom. 1952: 207 (54, 57) d.

Neoschongastia rattus, Gun. 1952: 32.

Euschongastia indica, in part, W. & F. 1952: 77 (ls).

Euschongastia rattus, Audy 1954*b*: 154 (t), Rad. 1954: 266.

Euschongastia (*Laurentella*) *rattus*, Audy 1956*a*: 2 (5) dtx.

Ascoschongastia (*Laurentella*) *rattus*, Dom. 1957*b* (tx), Wom. & A. 1957 (xl).

Records. NEW GUINEA (chigger identifications require confirmation). Rodent—*Rattus exulans, R. r. mindanensis, R. ringens*. AUSTRALIA. Rodent—*R. fuscipes assimilis*.

Ascoschongastia (?) raui Womersley

Schongastia (*Ascoschongastia*) *raui* Wom., 1952: 225 (64) D.
Euschongastia raui, Audy 1954*b*: 155 (t), Rad. 1954: 266.
Ascoschongastia (?) *raui*, Dom. 1957*b* (t), Audy & Wom. 1957 (dxl), Wom. & Audy 1957 (xl).
Records. AUSTRALIA. Reptile—*Lygosoma* (*Liolepisma*) *guichenoti*.

Ascoschongastia (Laurentella) roluis (Traub & Audy)

Euschongastia roluis Traub & A., 1954*b*: 79 (D), IMR 1952: 93 (1) (as no. 48).
Euschongastia (*Laurentella*) *roluis*, Audy 1956*a*: 13 (tx).
Ascoschongastia (*Laurentella*) *roluis*, Dom. 1957*b* (DNtx).
Records. BORNEO. Rodents—*Chiropodomys legatus*.

Ascoschongastia (Laurentella) sellnicki Audy & Womersley

Euschongastia n.sp. nr. *raui*, Audy 1954*b*: 155 (t), Har. 1954: 180 (b).
Euschongastia '*SELL*', Audy 1956*d*: 97 (dc).
Ascoschongastia (*Laurentella*) *sellnicki* Audy & Wom., 1957: ... D, Dom. 1957*b* (t), Har. 1957*b* (b).
Records. MALAYA. Rodent—*Rattus bowersi*, *R. mulleri*.

Ascoschongastia (Laurentella) soekaboemiensis (Takekawa)

Schongastia (*Ascoschongastia*) *soekaboemiensis*, Wom. 1952: 212 (56, 57) d.
Euschongastia soekaboemiensis, W. & F. 1952: 82, Audy 1954*b*: 154 (t), Rad. 1954: 266.
Euschongastia (*Laurentella*) *soekaboemiensis*, Audy 1956*a*: 13 (ctx).
Ascoschongastia (*Laurentella*) *soekaboemiensis*, Dom. 1957*b* (tx), Wom. & A. 1957 (xl).
Records. BURMA, South. Rodents—*Bandicota bengalensis*, *Rattus r. khyensis*. JAVA. Rodents—*R. r. diardi*, *R. jalorensis*.

Genus **Pseudoschongastia*** Lipovsky, 1951

Type: *Pseudoschongastia hungerfordi* Lipovsky, 1951

Pseudoschongastia novita Womersley & Audy

Pseudoschongastia novita Audy & Wom., 1957: ... (D).
Records. MALAYA. Rodent—*Rattus rajah*.

* Wharton & Fuller place *Pseudoschongastia* in the subfamily Gahrliepiinae (Walchiinae) but this is no longer acceptable. Audy (1956*a*) and Audy & Wom. (1957) discuss relationships.

Genus **Helenicula** Audy, 1954

Type: *Neoschongastia lanius* Radford, 1946

Helenicula comata (Womersley)

Schongastia (*Ascoschongastia*) *comata* Wom. 1952: 178 (43) D.
Euschongastia (*Helenicula*) *comata*, Audy 1954*b*: 152 (t).
Euschongastia comata, Rad. 1954: 264.
Helenicula comata, Wom. & A. 1957 (xl).
Records. INDIA, North. Rodent—'Rat'. PHILIPPINES. Unknown host.

Helenicula covelli (Radford)

Neoschongastia covelli Rad., 1953*a*: 233 (D).
Euschongastia covelli, Rad. 1954: 264.
Euschongastia (*Helenicula*) *covelli*, Audy 1954*b*: 153 (t).
Helenicula covelli, Wom. & A. 1957 (xl).
Records. INDIA, North-east. Rodent—*Callosciurus p. pygerythrus*.

Helenicula globulare (Walch)

Schongastia (*Ascoschongastia*) ? *globulare*, Wom. 1952: 174 (41) d.
Neoschongastia globulare, Gun. 1952: 27.
Euschongastia globularis (*-e*), W. & F. 1952: 76, Audy *et al.* 1953: 34 (c).
Euschongastia (*Helenicula*) *globulare*, Audy 1954*b*: 152 (t).
Helenicula globulare, Wom. & A. 1957 (xl).
Records (chigger identification provisional). ? INDIA, North-east. Rodent—*Rattus r. bullocki*.

Helenicula hongkongensis Womersley

Helenicula hongkongensis Wom., 1957 (D).
Record. HONG KONG. Rodent—*Rattus rattus* subsp.

Helenicula kohlsi (Philip & Woodward)

Schongastia (*Ascoschongastia*) *kohlsi*, Wom. 1952: 176 (42) d, 385 (101) DN.
Neoschongastia kohlsi, Gun. 1952: 33.
Euschongastia kohlsi, W. & F. 1952: 78, Audy *et al.* 1953: 34, 35 (bc), Rad. 1954: 265.
Euschongastia (*Helenicula*) *kohlsi*, Audy 1954*a*: 34 (bc), 1954*b*: 152 (t).
Helenicula kohlsi, Wom. & A. 1957 (xl).
Records. INDIA, North-east. Rodents—*Rattus r. bullocki*.

Helenicula lanius (Radford)

Schongastia (*Ascoschongastia*) *lanius*, Wom. 1952: 179 (43) d, 384 (102) DN.
Neoschongastia lanius, Gun. 1952: 33.
Euschongastia lanius, W. & F. 1952: 78, Audy *et al.* 1953: 34, 35 (bc), Rad. 1954: 265.

Euschongastia mutabilis, Audy 1947 (c), mis-identified.
Euschongastia (*Helenicula*) *lanius*, Audy 1954*a*: 34 (bc), 1954*b*: 152 (t).
Ascoschongastia mutabilis, Hammen 1956: 21, mis-identified.
Helenicula lanius, Wom. & A. 1957 (xl).
Records. INDIA, North-east (not South Burma). Rodents—*Rattus r. bullocki*.

Helenicula mutabilis (Gater)

Schongastia (*Ascoschongastia*) *mutabilis*, Wom. 1952: 180 (44) d, 386 (193) DN.
Neoschongastia mutabilis, Gun. 1952: 28.
Euschongastia mutabilis, W. & F. 1952: 79, Fuller 1952: 192 (dc), Rad. 1954: 265 (*not* Audy 1950 (c), see *H. lanius*).
Euschongastia (*Helenicula*) *mutabilis*, Audy 1954*b*: 152 (t), Audy 1956*d*: 96 (dc).
Helenicula mutabilis, Wom. & A. 1957 (xl), Har. 1957 (b).
Records. MALAYA. Carnivores—*Paradoxurus hermaphroditus*. (*Note*. Records for INDIA, Audy 1947: 295, Audy *et al.* 1953: 34, were based on mis-identified material, *vide* Fuller 1952: 194.)

Helenicula signata (Womersley)

Schongastia (*Ascoschongastia*) *signata* Wom. 1952: 174 (41) D.
Euschongastia (*Helenicula*) *signata*, Audy 1954*b*: 152 (t).
Euschongastia signata, Rad. 1954: 266.
Helenicula signata, Wom. & A. 1957 (xdc).
Records. BORNEO. Unattached; and on 'various hosts' (rodents, insectivores, carnivores, ungulates) to be published.

Genus Neoschongastia* Ewing, 1929

Type: *Schongastia americana* Hirst, 1921

Neoschongastia americana (Hirst)

Neoschongastia americana solomonis Wharton & Hardcastle, 1946.
Neoschongastia americana solomonis, Wom. 1952: 263 (74) d, 390 (106) dN (?), W. & F. 1952: 84, Rad. 1954: 263, Wom. & A. 1957 (l).
Neoschongastia solomonis, Gun. 1952: 35.

Neoschongastia backhousei (Gunther)

Neoschongastia backhousei, Wom. 1952: 253 (69) d, Gun. 1952: 29, W. & F. 1952: 84, Rad. 1954: 263, Wom. & A. 1957 (l).

* The following species, listed by Wharton & Fuller and by Womersley & Audy are omitted because they have been recorded only from islands outside the regions covered by the checklist; but since they are bird-chiggers they may be expected to occur within these regions: *atollensis*, *ewingi*, *monticola*, *namrui*, *pauensis*, *posekanyi*, *strongi*.

Neoschongastia bougainvillensis Wharton & Hardcastle

Neoschongastia bougainvillensis, Wom. 1952: 249 (74) d, Gun. 1952: 33, W. & F. 1952: 84, Rad. 1954: 263, Wom. & A. 1957 (l).

Neoschongastia carveri Wharton & Hardcastle

Neoschongastia carveri, Wom. 1952: 272 (75) d, 389 (106) dN, Gun. 1952: 34, W. & F. 1952: 85, Sasa & Jam. 1954: 270 (265) dc, Audy 1954*b*: 156 (t), Rad. 1954: 263, Wom. & A. 1957 (l).

Neoschongastia dubia Gunther

Neoschongastia dubia, Wom. 1952: 269 (74) d, Gun. 1952: 29, W. & F. 1952: 85, Rad. 1954: 263, Wom. & A. 1957 (l).

Neoschongastia egretta Gunther

Neoschongastia egretta, Wom. 1952: 254 (74) d, Gun. 1952: 34, W. & F. 1952: 85, Rad. 1954: 263, Wom. & A. 1957 (l).

Neoschongastia entomyza* Womersley

Neoschongastia entomyza Wom., 1952: 265 (72, 73) D, Rad. 1954: 263, Wom. & A. 1957 (l).

Records. AUSTRALIA. Birds—*Corvus cecilae, Cracticus torquatus, Entomyzon cyanotis, Gymnorhina tibicen, Ocyphaps lophotes, Struthidia cinerea.*

Neoschongastia gallinarum (Hatori)

Neoschongastia gallinarum, Wom. 1952: 252 (69) d, 390 (106) dN, Gun. 1952: 27, W. & F. 1952: 85, Rad. 1954: 263, Audy 1956*b*: 64 (c), 1956*d*: 97 (c), Wom. & A. 1957 (l).

Neoschongastia mcqueeni Womersley

Neoschongastia mcqueeni Wom., 1952: 260 (72) D, Rad. 1954: 263, Wom. & A. 1957 (l).

Records. OCEANIA, Solomon Is. Unrecorded host.

Neoschongastia owiensis Womersley

Neoschongastia owiensis Wom., 1952: 259 (71, 72) D, Rad. 1952: 263, Wom. & A. 1957 (l).

Records. NEW GUINEA and OCEANIA. Unattached.

Neoschongastia rectangulare (Womersley)

Schongastia (*Ascoschongastia*) *rectangulare* Wom., 1952: 186 (46) D.
Euschongastia rectangulare, Audy 1954*b*: 155, 156 (t), Rad. 1954: 266.
Neoschongastia rectangulare, Wom. & A. 1957 (xl).

* Very close to *N. thomasi* (Rad.), of which *entomyza* might be a synonym.

Neoschongastia retrocincta Gunther

Neoschongastia retrocincta, Wom. 1952: 256 (70) d, Gun. 1952: 30, W. & F. 1952: 86, Rad. 1952: 263, Wom. & A. 1957 (l).

Neoschongastia riversi Wharton & Hardcastle

Neoschongastia riversi, Wom. 1952: 271 (74) d, Gun. 1952: 34, Rad. 1954: 264, W. & F. 1952: 86, Audy 1954*b*: 156 (t), 1956*b*: 65 (c), 1956*d*: 97 (dc), Wom. & A. 1957 (xl).

Records. MALAYA. Reptiles—*Draco fimbriatus*, *D. maximus*.

Neoschongastia salmi (Oudemans)

Neoschongastia salmi, Wom. 1952: 267 (73) d, Fuller 1952: 179 (180, 181) d, Gun. 1952: 27, W. & F. 1952: 86, Rad. 1954: 264, Wom. & A. 1957 (l).

Neoschongastia struthidia Womersley

Neoschongastia struthidia Wom., 1952: 255 (70) D.

Records. AUSTRALIA. Bird—*Struthidia cinerea*.

Neoschongastia thomasi (Radford)

Neoschongastia thomasi, Wom. 1952: 266 (73) d, Gun. 1952: 33, W. & F. 1952: 87, Audy *et al.* 1953: 29, 34 (c), Rad. 1954: 264, Wom. & A. 1957 (xl).

Neoschongastia entomyza, *q.v.* (? *syn*).

Records. INDIA, North-east. Birds—'Babbler', 'Hawk', 'Laughing Thrush', *Rhodophila ferrea*, *Saxicola capiata*.

Neoschongastia yeomansi Gunther

Neoschongastia yeomansi, Wom. 1952: 257 (71) d, Gun. 1952: 30, W. & F. 1952: 87, Rad. 1954: 264, Wom. & A. 1957 (xl).

Genus **Mackiena** Traub & Evans, 1950

Type: *Mackiena empodiformia* Traub & Evans, 1950

Mackiena empodiformia Traub & Evans

Mackiena empodiformia (*-is*), Wom. 1952: 278 (68) d, Gun. 1952: 37, W. & F. 1952: 278 (68) d, Audy 1954*b*: 156 (t), Rad. 1954: 262, Wom. & A. 1957.

Genus **Traubacarus*** Audy & Nadchatram, 1957

Type: *Traubacarus vercammeni* Audy & Nadchatram, 1956

Traubacarus alticola Audy & Nadchatram

Traubacarus alticola Audy & Nad., 1957*a*: ... D.
Records. MALAYA. Rodent—*Rattus alticola*.

Traubacarus brachypus Audy & Nadchatram

Traubacarus brachypus Audy & Nad., 1957*a*: ... (D).
Records. MALAYA. Rodents—*Rattus rajah pellax, R. rajah surifer, R. whiteheadi*.

Traubacarus browningi Audy & Nadchatram

Traubacarus browningi Audy & Nad., 1957*a*: ... (D).
Records. MALAYA. Rodents—*Rattus bowersi*.

Traubacarus domrowi Audy & Nadchatram

Traubacarus domrowi Audy & Nad., 1957*a*: ... (D).
Records. MALAYA. Rodents—*Rattus bowersi, R. rajah surifer, R. sabanus*. BORNEO. Rodents—*Rattus alticola, R. rajah, R. whiteheadi*.

Traubacarus harrisoni Audy & Nadchatram

Traubacarus harrisoni Audy & Nad., 1957*a*: ... D.
Records. MALAYA. Rodents—*Rattus bowersi, R. jalorensis, R. mulleri, R. rajah, R. sabanus, R. whiteheadi*.

Traubacarus intermedia Audy & Nadchatram

Traubacarus intermedia Audy & Nad., 1957*a*: ... (D), Har. 1957 (b).
Records. MALAYA. Rodents—*Rattus bowersi*.

Traubacarus jadini Audy & Nadchatram

Traubacarus jadini Audy & Nad., 1957*a*: ... D.
Records. MALAYA. Rodents—*Rattus bowersi, R. edwardsi, R. rajah, R. sabanus*.

Traubacarus manipurensis (Radford)

Schongastia (*Ascoschongastia*) *manipurensis*, Wom. 1952: 204 (53) d.
Neoschongastia manipurensis, Gun. 1952: 33.
Doloisia manipurensis, W. & F. 1952: 72, Audy *et al.* 1953: 34 (c), Rad. 1954: 267.
Doloisia (*VN*-group) *manipurensis*, Audy 1954*b*: 159 (dtx).

* This genus of intranasal chiggers is closely related to *Doloisia* and is the *VN*-group of Audy 1954*b*: 157; it is divided into two distinct groups, the long-legged *vercammeni*-group (*alticola, harrisoni, intermedia, jadini, manipurensis, owenevansi, varmai, vercammeni*) and the *brachypus*-group (*brachypus, browningi, domrowi*). The species of *Traubacarus* were provisionally listed by Audy (1956*d*: 98 dc) as a 'new genus'.

Traubacarus manipurensis, Audy & Nad. 1957*a*: ... (dx), Wom. & A. 1957 (xl).
Records. INDIA, North-east. Rodents—?? *Rattus r. bullocki* (probably *R. bowersi*).

Traubacarus owenevansi Audy & Nadchatram

Traubacarus owenevansi Audy & Nad., 1957*a*: ... (D).
Records. MALAYA. Rodents—*Rattus rajah*.

Traubacarus varmai Audy & Nadchatram

Traubacarus varmai Audy & Nad., 1957*a*: ... (D).
Records. MALAYA. Rodents—*Rattus bowersi*, *R. rajah*, *R. whiteheadi*.

Traubacarus vercammeni Audy & Nadchatram

Traubacarus vercammeni Audy & Nad., 1957*a*: ... (D).
Records. MALAYA. Rodents—*Rattus bowersi*, *R. rajah pellax*, *R. rajah surifer*, *R. sabanus*.

Genus **Schoutedenichia** Jadin & Vercammen-Grandjean, 1954

Type: *Schoutedenichia fulleri* Jadin & Vercammen-Grandjean, 1954

Schoutedenichia jubbulporensis (Womersley)

Trombicula jubbulporensis Wom., 1952: 119 (20) D, Audy 1954*b*: 160 (t), Rad. 1954: 257.
Schoutedenichia jubbulporensis, Audy 1956*c*: 84 (dtx), Verc. 1957 (dt).
Records. INDIA, Peninsular. Rodent—'Rat'.

Schoutedenichia nausheraensis (Womersley)

Schongastia (*Ascoschongastia*) *nausheraensis* Wom., 1952: 202 (52) D.
Doloisia (*crocidura*-group) *nausheraensis*, Audy 1954: 160 (tx).
Euschongastia nausheraensis, Rad. 1954: 265.
Schoutedenichia nausheraensis, Audy 1956*c*: 84 (dtx), Verc. 1957 (dt).
Records. INDIA, North. Rodent—'Mouse'.

Schoutedenichia vercammeni Audy

Schoutedenichia vercammeni Audy, 1956*c*: 82 (D), Har. 1957 (b), Verc. 1957 (dt).
Records. MALAYA. Rodent—*Rattus sabanus*.

Genus **Guntherana** Womersley & Heaslip, 1943

Type: *Neoschongastia kallipygos* Gunther, 1939

Guntherana kallipygos (Gunther)

Guntherana kallipygos Gun. 1952: 2, W. & F. 1952: 83, Audy 1954*b*: 156, Wom. & A. 1957 (xl).
Guntherana bipygalis, Wom. 1952: 245 (67) d, 373 (99) DN, Rad. 1954: 267, synonym.

Guntherana parana, W. & F. 1952: 83, Rad. 1954: 267, synonym.
Records (of *bipygalis*, synonym, omitted by W. & F. 1952). NEW GUINEA. Rodents—*Melomys monktoni*, *M. rufescens stalkeri*, *Melomys* sp., *Rattus exulans browni*, *R. ruber mordax*, *R. ruber ringens*, *Uromys caudimaculatus aruensis*. Marsupials—*Echymipera k. kalubu*, *Peroryctes raffrayanus*. Also unattached. AUSTRALIA. Rodents—*Rattus culmorum youngi*. Marsupials—*Thylacis torosus*.

Guntherana tindalei (Womersley)

Guntherana tindalei, Wom. 1952: 374 (99) dNx, W. & F. 1952: 83, Audy 1954*b*: 157 (t), Wom. & A. 1957.
Trombicula tindalei, Gun. 1952: 22.

Guntherana translucens (Womersley)

Guntherana translucens, Wom. 1952: 375 (100) dA, Audy 1954*b*: 157 (t), Wom. & A. 1957.
Trombicula translucens, Gun. 1952: 22.
Trombicula (*Trombicula*) *translucens*, W. & F. 1952: 71.

Subfamily GAHRLIEPIINAE Womersley, = WALCHIINAE Ewing, *sensu stricto*

Genus **Gahrliepia** Oudemans, 1912

Type: *Typhlothrombidium nanus* Oudemans, 1940

Subgenus **Gahrliepia** Oudemans, 1912

Type: *Typhlothrombidium nanus* Oudemans, 1940

Gahrliepia (Gahrliepia) ampullata Traub & Morrow

Gahrliepia (*Gahrliepia*) *ampullata* Traub & M., 1955: 43 (D).
Records. BORNEO, North. Rodents—*Rattus cremoriventer*, *R. w. whiteheadi*.

Gahrliepia (Gahrliepia) barbigera Traub & Morrow

Gahrliepia (*Gahrliepia*) *barbigera* Traub & M., 1957: ... D.
Records. INDIA, North. Insectivore—Shrews.

Gahrliepia (Gahrliepia) cetrata Gater

Gahrliepia (*Gahrliepia*) *cetrata*, Wom. 1952: 304 (82) d, Audy 1954*b*: 162, 1956*d*: 98 (dc), Traub & M., 1955: 59 (D), Har. 1957 (b), Wom. & A. 1957 (xl).
Gahrliepia cetrata, Gun. 1952: 46, W. & F. 1952: 93, Rad. 1954: 270.
Records. MALAYA. Rodents—*Rattus edwardsi*, *R. mulleri*, *R. sabanus*.

Gahrliepia (Gahrliepia) ciliata Gater

Gahrliepia (*Gateria*) *ciliata*, Wom. 1952: 309 (83) d.
Gateria ciliata, Gun. 1952: 47, W. & F. 1952: 93, Rad. 1954: 270.
Gahrliepia (*Gahrliepia*) *ciliata*, Audy 1954*b*: 162, 1956*d*: 98 (cd), Traub & M. 1955: 68 (d), Wom. & A. 1957 (l).
Records. MALAYA. Carnivore—*Prionodon linsang*.

Gahrliepia (Gahrliepia) crocidura (Radford)

Gahrliepia (*Gahrliepia*) *crocidura*, Traub & M. 1955: 71 (73) ds, Wom. & A. 1957 (xl).
Gahrliepia (*Gateria*) *crocidura*, Wom. 1952: 307 (82) d, Audy 1954*b*: 162.
Gahrliepia (*Gateria*) *lancearia*, Wom. 1952: 306 (82) d.
Gahrliepia (*Gateria*) *lancearia*, Wom. 1952: 306 (82) d, synonym.
Gateria crocidura, Gun. 1952: 47, W. & F. 1952: 93, Rad. 1954: 270.
Gateria lancearia, Gun. 1952: 48, W. & F. 1952: 94, Rad. 1954: 270.
Records (for *G. lancearia*, syn., acquired). INDIA, North-east. Insectivore—*Talpa micrura*.

Gahrliepia (Gahrliepia) darita Traub & Morrow

Gahrliepia (*Gahrliepia*) *darita* Traub & M., 1955: 12 (D).
Records. ASSAM. Insectivore—*Suncus* sp. BURMA, North. Rodents—*Rattus f. fulvescens*, *R. r. sladeni*. Insectivores—*Crocidura* sp., *Tupaia glis versurae*, *Suncus* sp.

Gahrliepia (Gahrliepia) decora Womersley

Gahrliepia (*Gahrliepia*) *decora* Wom., 1952: 303 (80) D, Audy 1954*b*: 162, 1956*d*: 98 (cd), Traub & M. 1955: 64, Wom. & A. 1957 (xl).
Records. MALAYA. Rodents—*Rattus sabanus*.

Gahrliepia (Gahrliepia) dupliseta Traub & Morrow

Gahrliepia (*Gahrliepia*) *dupliseta* Traub & M. 1955: 26 (D).
Records. INDIA, North-east (Assam). Insectivore—*Suncus murinus fulvocinereus*.

Gahrliepia (Gahrliepia) elbeli Traub & Morrow

Gahrliepia (*Gahrliepia*) *elbeli* Traub & M. 1955: 18 (D).
Records. THAILAND. Rodent—*Menetes berdmorei*.

Gahrliepia (Gahrliepia) evansi Traub & Morrow

Gahrliepia (*Gahrliepia*) *evansi* Traub & M., 1955: 30 (D).
Records. BURMA, North. Rodents—*Rattus r. sladeni*, *Rattus* sp.

Gahrliepia (Gahrliepia) exilis Traub & Morrow

Gahrliepia (*Gahrliepia*) *exilis* Traub & M., 1955: 6 (D).
Records. BURMA, North. Rodents—*Rattus f. fulvescens*, *R. r. sladeni*. Insectivores—*Tupaia glis versurae*.

Gahrliepia (Gahrliepia) fenestrulata Traub & Morrow

Gahrliepia (Gahrliepia) fenestrulata Traub & M., 1957: ... D.

Records. THAILAND. Rodents—'Rat', *Menetes.*

Gahrliepia (Gahrliepia) fimbriata Traub & Morrow

Gahrliepia (Gahrliepia) fimbriata Traub & M., 1955: 10 (D).

Records. BURMA, North. Rodent—*Rattus r. yunnanensis.*

Gahrliepia (Gahrliepia) fletcheri Gater

Gahrliepia (Gateria) fletcheri, Wom. 1952: 311 (83) d, Audy 1954*b*: 162 (t).

Gahrliepia (Gateria) spinulosa, Wom. 1952: 309 (83) d, Audy 1954*b*: 162 (t), synonym.

Gateria fletcheri, Gun. 1952: 48, W. & F. 1952: 94, Rad. 1954: 270.

Gateria spinulosa, Gun. 1952: 48, W. & F. 1952: 94, Rad. 1954: 270, synonym.

Gahrliepia (Gahrliepia) fletcheri, Traub & M., 1955: 55 (dct), Audy 1956*d*: 98 (dc), Wom. & A. 1957 (xl), Har. 1957 (b).

Records. MALAYA. Rodents—*Rattus argentiventer, R. bowersi, R. canus, R. exulans concolor, R. mulleri.* Insectivore—*Tupaia minor.*

Gahrliepia (Gahrliepia) gemina Traub & Morrow

Gahrliepia (Gahrliepia) gemina Traub & M., 1955: 23 (D).

Records. BURMA, North. Rodents—*Rattus f. fulvescens, R. r. sladeni*, Rattus sp. Insectivore—*Crocidura russula vorax.*

Gahrliepia (Gahrliepia) granulata Traub & Morrow

Gahrliepia (Gahrliepia) granulata Traub & M., 1955: 50 (D).

Records. BORNEO. Rodents—*Rattus a. alticola, R. w. whiteheadi, Rattus* sp.

Gahrliepia (Gahrliepia) hirsuta (Radford)

Gahrliepia (Gateria) hirsuta, Wom. 1952: 305 (82) d, 398 (109) DN, Audy 1954*b*: 162 (t).

Gateria hirsuta, Gun. 1952: 47, W. & F. 1952: 94, Rad. 1954: 270.

Gahrliepia (Gahrliepia) hirsuta, Traub & M. 1955: 61 (d), Wom. & A. 1957 (xl).

Records. BURMA, South. Insectivore—*Talpa micrura.*

Gahrliepia (Gahrliepia) insigne Womersley

Gahrliepia (Gahrliepia) insigne Wom., 1952: 302 (79), Audy 1954*b*: 162 (t), 1956*d*: 98 (cd), Traub & M., 1955: 66 (65) dc, 1957 (c), Wom. & A. 1957 (xl), Har. 1957 (b).

Gahrliepia insigne, Rad. 1954: 270.

Records. MALAYA. Rodents—*Rattus alticola, R. bowersi, R. edwardsi, R. rajah, R. sabanus.* Insectivore—*Tupaia glis.*

Gahrliepia (Gahrliepia) laciniata Traub & Morrow

Gahrliepia (*Gahrliepia*) *laciniata* Traub & M., 1955: 37 (D).
Records. BORNEO. Rodents—*Rattus a. alticola, R. rajah, R. rapit, R. sabanus.*

Gahrliepia (Gahrliepia) longipili (Radford)

Gahrliepia (*Gateria*) *longipilis*, Wom. 1952: 307 (82) d, Audy 1954*b*: 162 (t).
Gateria longipili, Gun. 1952: 48, W. & F. 1952: 94, Rad. 1954: 270.
Gahrliepia (*Gahrliepia*) *longipili*, Traub & M., 1955: 72, Wom. & A. 1957 (xl).

Gahrliepia (Gahrliepia) marshi Traub & Morrow

Gahrliepia (*Gahrliepia*) *marshi* Traub & M., 1957: ... D.
Records. THAILAND. Ungulate—Wild Pig.

Gahrliepia (Gahrliepia) neterella Traub & Morrow

Gahrliepia sp. nr. *fletcheri*, Har. 1954: 181 (b).
Gahrliepia (*Gahrliepia*) *neterella* Traub & M., 1955: 14 (D), 1957 (c), Audy 1956*d*: 99 (cd), Har. 1957 (b).
Records. MALAYA. Rodents—*Rattus bowersi, R. edwardsi, R. mulleri, R. sabanus.*

Gahrliepia (Gahrliepia) ordinata Traub & Morrow

Gahrliepia (*Gahrliepia*) *ordinata* Traub & M., 1957: ... D.
Records. BORNEO. Rodents—*Rattus alticola.*

Gahrliepia (Gahrliepia) ornata Womersley

Gahrliepia (*Gahrliepia*) *ornata* Wom., 1952: 304 (81) D, 400 (115) DN, Audy 1954*b*: 162 (t), 1956*d* (dc), Traub & M., 1955: 63.
Gahrliepia sp. nr. *decora*, Harrison 1954: 181 (b).
Gahrliepia ornata, Rad. 1954: 270.
Records. MALAYA. Rodents—*Rattus mulleri, R. sabanus.*

Gahrliepia (Gahrliepia) penetrans Traub & Morrow

Gahrliepia (*Gahrliepia*) *penetrans* Traub & M., 1955: 45 (Db).
Records. BORNEO. Rodents—*Rattus w. whiteheadi.*

Gahrliepia (Gahrliepia) picta Traub & Morrow

Gahrliepia (*Gahrliepia*) *picta* Traub & M., 1955: 32 (D), Audy 1956*d*: 98 (cd), Har. 1957 (b).
Records. MALAYA. Rodents—*Rattus bowersi, R. sabanus.*

Gahrliepia (Gahrliepia) plurisetae Traub & Morrow

Gahrliepia (*Gahrliepia*) *plurisetae* Traub & M., 1955: 28 (D).
Records. INDIA, North-east. Insectivore—*Suncus murinus fulvocinereus.*

Gahrliepia (Gahrliepia) romeri Womersley

Gahrliepia (*Gateria*) *romeri* Wom., 1952: 308 (83) D, Audy 1954*b*: 162 (t).

Gahrliepia (*Gahrliepia*) *romeri*, Audy 1956*d* (dc), Traub & M. 1955: 74 (d), Wom. & A. 1957 (xl).

Records. HONG KONG. Rodents—*Rattus r. sladeni*, *R. rattus* subsp.

Gahrliepia (Gahrliepia) rutila Gater

Gahrliepia (*Gahrliepia*) *rutila*, Traub & M. 1955: 70 (dc), 1957 (c), Auyd 1956*d*: 99 (cd), Wom. & A. 1957 (xl).

Gahrliepia (*Gateria*) *rutila*, Wom. 1952: 308 (83) d, Audy 1954*b*: 162 (t).

Gateria rutila, Gun. 1952: 47, W. & F. 1952: 94, Rad. 1954: 270.

Records. MALAYA. Insectivore—*Tupaia glis.* Rodents—*Rattus bowersi*, *R. jalorensis.*

Gahrliepia (Gahrliepia) tenella Traub & Morrow

Gahrliepia (*Gahrliepia*) *tenella* Traub & M., 1955: 16 (D).

Records. BURMA, North. Rodent—*Rattus r. yunnanensis.*

Gahrliepia (Gahrliepia) tessellata Traub & Morrow

Gahrliepia (*Gahrliepia*) *tessellata* Traub & M., 1955: 34 (D), Audy 1956*d*: 98 (cd).

Records. MALAYA. Rodent—*Lariscus insignis.* Insectivore—*Hylomys suillus.*

Gahrliepia (Gahrliepia) tuberculata Traub & Morrow

Gahrliepia (*Gahrliepia*) *tuberculata* Traub & M., 1955: 40 (D).

Records. BORNEO. Rodents—*Rattus w. whiteheadi*, *R. mulleri*, *R. rajah*, *R. sabanus*, *Rattus* spp.

Gahrliepia (Gahrliepia) tylana Traub & Morrow

Gahrliepia (*Gahrliepia*) *tylana* Traub & M., 1955: 21 (D).

Records. THAILAND. Rodents—*Bandicota* sp. (*B.* ? *bengalensis*), *Menetes berdmorei.*

Subgenus **Schongastiella** Hirst 1915

Type: *Schongastiella bengalensis* Hirst 1915

Gahrliepia (Schongastiella) argalea Traub & Morrow

Gahrliepia (*Schongastiella*) *argalea* Traub & M., 1957: ... D.

Records. MALAYA. Rodent—*Rattus sabanus.* Insectivore—*Tupaia glis.*

Gahrliepia (Schongastiella) arona Traub & Evans

Gahrliepia (*Schongastiella*) *arona* Traub & E., 1954: 95 (97) D, Audy 1954*b*: 162 (t), 1956*d*: 99 (dc), Wom. & A. 1957: ... (dN), Har. 1957 (b).

Schongastiella arona, Harrison 1954: 181 (b).

Records. MALAYA. Rodents—*Rattus mulleri*, *R. whiteheadi.*

Gahrliepia (Schongastiella) bengalensis (Hirst)

Gahrliepia (*Schongastiella*) *bengalensis*, Wom. 1952: 298 (78) d, Audy 1954*b*: 162 (t), Wom. & A. 1957 (xl).

Schongastiella bengalensis, Audy 1952: 150 (t), W. & F. 1952: 94, Gun. 1952: 46, Rad. 1954: 271.

Records. INDIA, North. Rodent—'Rat'.

Gahrliepia (Schongastiella) birella Traub & Evans

Gahrliepia (*Schongastiella*) *birella* Traub & E., 1954: 95 (96) D, Audy 1954*b*: 162 (l), 1956*d*: 99 (dc).

Records. MALAYA. Rodents—*Rattus bowersi*.

Gahrliepia (Schongastiella) brevis (Radford)

Gahrliepia (*Schongastiella*) *brevis*, Wom. 1952: 298 (78) d, Audy *et al.* 1953: 150 (t), Audy 1954*b*: 162 (t), Wom. & A. 1957 (xl).

Schongastiella brevis, Gun. 1952: 46, W. & F. 1952: 94, Audy *et al.* 1953: 35 (c), Rad. 1954: 270.

Gahrliepia (Schongastiella) ceylonica Womersley

Gahrliepia (*Schongastiella*) *ceylonica* Wom., 1952: 295 (77) D, 399 (109) DN, Audy 1954*b*: 162 (t), Traub & E. 1954: 98 (t), Wom. & A. 1957 (xl).

Records. INDIA, North. Rodent—'Mouse'. CEYLON. Rodents—*Mus cervicolor fulvidiventris*, *Rattus r. kandiyanus*.

Gahrliepia (Schongastiella) cucurbitula Traub & Morrow

Gahrliepia (*Schongastiella*) *cucurbitula* Traub & M., 1957: ... D.

Records. BORNEO. Rodents—*Rattus mulleri*, *R. w. whiteheadi*.

Gahrliepia (Schongastiella) erula Traub & Evans

Gahrliepia (*Schongastiella*) *erula* Traub & E., 1954: 91 (D), Audy 1954*b*: 162 (t)

Records. BURMA, North. Rodents—*Rattus f. fulvescens*, *R. r. sladeni*, *R. r. yunnanensis*.

Gahrliepia (Schongastiella) gammonsi Traub & Evans

Gahrliepia (*Schongastiella*) *gammonsi* Traub & E., 1954: 100 (101) D, Audy 1954*b*: 162 (t).

Records. INDIA, North-east. Insectivore—*Anourosorex s. squamipes*.

Gahrliepia (Schongastiella) helata Traub & Evans

Gahrliepia (*Schongastiella*) *helata* Traub & E., 1954: 93 (D), Audy 1954*b*: 162 (t).

Records. BURMA, North. Insectivore—*Crocidura* sp.

Gahrliepia (Schongastiella) hipposideros (Audy)

Schongastiella hipposideros Audy, 1952: 148 (D).

Gahrliepia (*Schongastiella*) *hipposideros*, Traub & E. 1954: 105 (t), Audy 1954*b*: 162 (t), 1956*d* (dc).

Records. MALAYA. Bat—*Hipposideros* sp.

Gahrliepia (Schongastiella) homunguis Abdussalam

Schongastiella homunguis, W. & F. 1952: 94.

Gahrliepia (Schongastiella) kalrata Traub & Evans

Gahrliepia (*Schongastiella*) *kalrata* Traub & E., 1954: 98 (D).

Records. INDIA, North. Rodent—*Rattus rattoides* ? *turkestanicus*, 'Mouse'.

Gahrliepia (Schongastiella) kumaonensis Womersley

Gahrliepia (*Schongastiella*) *kumaonensis* Wom., 1952: 300 (78) D, Audy 1952: 150 (t), 1954*b*: 162 (t).

Schongastiella kumaonensis, Rad. 1954: 271.

Records. INDIA, North. Insectivore—'Shrew'.

Gahrliepia (Schongastiella) ligula (Radford)

Gahrliepia (*Schongastiella*) *ligula*, Wom. 1952: 296 (77) d, 399 (108) DN, Audy *et al.* 1953: 36, 37 (bd), Traub & E. 1954: 100 (bdtc), Wom. & A. 1957 (xl).

Schongastiella sp., Audy 1947: 295 (bc).

Schongastiella ligula Gun. 1952: 46, W. & F. 1952: 94, Rad. 1954: 271.

Records. BURMA, North and INDIA, North-east (Assam). Rodents—*Bandicota* sp., *Mus musculus homourus*, *Rattus manipulus*, *R. r. sladeni*, *R. r. yunnanensis*. Insectivores—*Anourosorex* sp., *Crocidura* sp., *Suncus* sp., *Tupaia glis versurae*. BURMA, South. Rodents—*Bandicota bengalensis*, *Rattus r. khyensis*. INDIA, North. Rodents and Insectivores—Common on 'Rats', 'Shrews'. INDIA, North-east. Rodents—*Callosciurus* sp., *Rattus manipulus*, *R. r. bullocki*, *Rattus* sp. Insectivores—*Anourosorex squamipes*, *Suncus* ? *murinus*.

Gahrliepia (Schongastiella) liota Traub & Evans

Gahrliepia (*Schongastiella*) *liota* Traub & E., 1954: 89 (D), Audy 1954*b*: 162 (t).

Records. INDIA, North-east. Insectivores—*Anourosorex s. squamipes*, *Crocidura* sp., *Suncus murinus fulvocinereus*, *Suncus* sp.

Gahrliepia (Schongastiella) punctata (Radford)

Gahrliepia (*Schongastiella*) *punctata*, Wom. 1952: 299 (78) d, Audy 1952: 150 (t), 1954*b*: 162 (t), Audy *et al.* 1953: 36 (c), Traub & E. 1954: 91 (t), 103 (dc), Wom. & A. 1954 (xl).

Schongastiella punctata, Gun. 1952: 47, W. & F. 1952: 94, Rad. 1954: 271.

Records. INDIA, North-east (Assam) and BURMA, North. Rodents—*Mus musculus homourus, Rattus r. sladeni, R. r. yunnanensis,* 'Rat'. Insectivores (chief hosts)—*Anourosorex, Crocidura, Suncus.* INDIA, North-east (Manipur). Rodents—'Mouse' *Rattus r. bullocki,* 'Rats'. Insectivores—'Shrews'. INDIA, Peninsular. Rodents—'Rats'. CEYLON. Rodent—*Millardia meltada meltada.*

Subgenus **Walchia*** Ewing, 1931

Type: (*Thrombidium glabrum* Walch, 1927) = *Walchia ewingi* Fuller, 1952

Gahrliepia (Walchia) brennani Womersley

Gahrliepia (*Walchia*) *brennani* Wom., 1952: 294 (112) D, Audy 1954*b*: 161 (t), 1956*d*: 99 (dc), Traub & E. 1957: ... (dtc), Wom. & A. 1957 (xl), Har. 1957 (b).

Walchia brennani, Rad. 1954: 270.

Records. MALAYA. Rodents—*Rattus bowersi, Rhinosciurus laticaudatus.* Insectivores—*Tupaia glis.*

Gahrliepia (Walchia) cuspidata Traub & Evans

Gahrliepia (*Walchia*) *cuspidata* Traub & E., 1957: ... (D).

Records. MALAYA. Rodent—*Rattus sabanus.*

Gahrliepia (Walchia) disparunguis (Oudemans)

(1) Gahrliepia (Walchia) disparunguis disparunguis (Oudemans)

Gahrliepia (*Walchia*) *disparunguis,* Wom. 1952: 290 (76) d, in part.

Walchia disparunguis, Gun. 1952: 44, Fuller 1952: 207 (dt), W. & F. 1952: 92, Rad. 1954: 270.

Gahrliepia (*Walchia*) *disparunguis disparunguis,* Traub & E. 1957: ... (dtc).

Records. BURMA. Rodents—*Bandicota bengalensis, Rattus r. khyensis.*

(2) Gahrliepia (Walchia) disparunguis pingue (Gater)

Walchia pingue, W. & F. 1952: 92 (c).

Gahrliepia (*Walchia*) *disparunguis,* Wom. 1952: 290 (dc), in part.

Gahrliepia (*Walchia*) *disparunguis pingue,* Traub & E. 1957: ... (dtc), full documentation; Har. 1957 (b).

Records. CEYLON (?) (*disparunguis* of Womersley). Rodents—*Millardia meltada, Rattus r. kandiyanus.* MALAYA. Rodents—*Lariscus insignis, Rattus edwardsi, R. f. fulvescens, R. rajah, R. sabanus, R. whiteheadi, Rattus* sp.

Gahrliepia (Walchia) enode (Gater)

Gahrliepia (*Walchia*) *enode,* Wom. 1952: 293 (76) d, 397 (108) DN, Audy 1954*b*: 161 (t), 1956*d*: 99 (dc), Traub & E. 1957: ... (dtc), Wom. & A. 1957 (xl), Har. 1957 (b).

Walchia enode, W. & F. 1952: 92, Fuller 1952: 204 (dtkc), Rad. 1954: 270.

Walchia (?) *enode,* Gun. 1952: 44.

Records. ? BURMA, South (*enode* of Womersley). Rodents—*Bandicota bengalensis, Rattus r. khyensis.*

* Five species of *Walchia* were added to Traub & Evans's paper after this checklist went to press: Malaya—*alpestris, cupa, simulata*; Borneo—*mima, rectifunda.*

Gahrliepia (Walchia) erana Traub & Evans

Gahrliepia (*Walchia*) *erana* Traub & E., 1957: ... (D).

Records. BURMA, North. Rodents—*Rattus f. fulvescens, R. r. sladeni, R. r. yunnanensis.* Insectivores—*Anourosorex s. squamipes, Tupaia glis versurae.*

Gahrliepia (Walchia) ewingi (Fuller)

(1) **Gahrliepia (Walchia) ewingi ewingi (Fuller)**

Trombidium glabrum Walch, 1927: 926, preoccupied.

Gahrliepia (*Walchia*) *ewingi*, Wom. 1952: 292 (76) d.

Gahrliepia (*Walchia*) *pingue*, Wom. 1952: 293, Audy 1954*b*: 161 (t), not *pingue* Gater.

Walchia pingue, W. & F. 1952: 92, not *pingue* Gater.

Walchia glabrum, Gun. 1952: 44 (s), Fuller 1952: 204 (k), Rad. 1954: 270.

Gahrliepia (*Walchia*) *ewingi ewingi*, Traub & E. 1957: ... (dtc).

Records. BURMA, North. Rodents—*Rattus f. fulvescens, R. r. sladeni, R. r. flavipectus.* Insectivores—*Crocidura* sp., *Tupaia* sp. BORNEO. Rodents—*Rattus mulleri* (distinct form of *ewingi*). INDONESIA, Batavia (unchecked). Rodent—*Rattus r. diardi.*

(2) **Gahrliepia (Walchia) ewingi lupella** Traub & Evans

Gahrliepia (*Walchia*) *ewingi lupella* Traub & E., 1957: ... (D).

Records. THAILAND. Rodents—*Bandicota indica, Rattus rattus* sp. Carnivore—'Mongoose'.

Gahrliepia (Walchia) kritochaeta Traub & Evans

Gahrliepia (*Walchia*) *kritochaeta* Traub & E., 1957: ... (D).

Records. THAILAND. Rodent—*Rattus r. thai.*

Gahrliepia (Walchia) lewthwaitei (Gater)

Gahrliepia (*Walchia*) *lewthwaitei*, Wom. 1952: 289 (76) d, 397 (107) DN, Audy 1954: 161 (t), 1956*d*: 99 (dc), Traub & E. 1957: ... (dtc), Wom. & A. 1957 (xl), Har. 1956*a*, *b* (c), 1957 (b).

Walchia lewthwaitei, Fuller 1952: 204 (k), Gun. 1952: 45, W. & F. 1952: 92, Rad. 1954: 270.

Records. MALAYA. Rodents—*Rattus argentiventer, R. exulans, R. jalorensis, R. rajah, R. sabanus, R. whiteheadi.*

Gahrliepa (Walchia) manipurensis (Sinha)

Walchia manipurensis Sinha, 1954: 335 (D), 1947.

Gahrliepia (*Walchia*) *manipurensis*, Traub & E. 1957: ... (d), Sinha, 1957 (xl).

Records. INDIA, North-east. Rodent—*Bandicota bengalensis.*

Gahrliepia (Walchia) micropelta Traub & Evans

Gahrliepia (*Walchia*) *micropelta* Traub & E., 1957: ... (D).

Records. THAILAND. Rodents—*Mus* sp., *Rattus* sp.

Gahrliepia (Walchia) morobensis (Gunther)

Gahrliepia (*Walchia*) *morobensis*, Wom. 1952: 285 (76) d, Audy 1954: 161 (t), Traub & E. 1957 (dtc), Wom. & A. 1957 (xl).

Walchia morobensis, Fuller 1952: 205 (k), Gun. 1952: 45, Rad. 1954: 270, W. & F. 1952: 92.

Records. BURMA, South. Rodents—*Bandicota bengalensis, Rattus r. khyensis.*

Gahrliepia (Walchia) morrowae Traub & Evans

Gahrliepia (*Walchia*) *morrowae* Traub & E., 1957: ... (D).

Records. BORNEO. Rodents—*Rattus alticola, R. sabanus.*

Gahrliepia (Walchia) naniparma Traub & Evans

Gahrliepia (*Walchia*) *naniparma* Traub & E., 1957: ... (D).

Records. BORNEO. Rodent—*Rattus whiteheadi.*

Gahrliepia (Walchia) rioi (Gunther)

Gahrliepia (*Walchia*) *rioi*, Wom. 1952: 295 (77) d, Audy 1954*b*: 161 (t), Traub & E. 1957: ... (dt), Wom. & A. 1957 (xl).

Walchia rioi, W. & F. 1952: 92, Rad. 1954: 270.

Walchia (?) *rioi*, Gun. 1952: 45.

Gahrliepia (Walchia) rustica Gater

Gahrliepia (*Walchia*) *rustica*, Wom. 1952: 286 (76) d, 396 (107) DN, Audy 1954*b*: 161 (t), 1956*d*: 100 (dc), Traub & E. 1957: ... (dtc), Wom. & A. 1957 (xl), Har. 1957 (b).

Gahrliepia (*Walchia*) *turmalis*, in part, Wom. 1952: 286, mis-identified.

Walchia rustica, Gun. 1952: 45, W. & F. 1952: 93, Rad. 1954: 270.

Records. BURMA, North and INDIA, North-east. Rodents—*Rattus n. nitidus, R. r. sladeni, R. r. yunnanensis.* Insectivores—*Crocidura* sp., *Suncus murinus fulvocinereus.* Carnivore—*Panthera pardus* subsp. Bird—*Cyanops lineatus hodgsoni.* BURMA, South (*turmalis* of Womersley, *err. det.*). Rodents—*Bandicota bengalensis, Rattus exulans concolor, R. r. khyensis.* THAILAND. Rodents—*Bandicota bengalensis, Cannomys badius minor, Menetes berdmorei, Rattus* (? *R. rattus* subsp.). HONG KONG. Rodents—*Bandicota indica nemorivaga, Rattus r. sladeni, R. rattus* subsp. MALAYA. Rodents—*Callosciurus notatus, Rattus mulleri, R. r. jarak, R. sabanus.* INDONESIA, Malacca Straits. Rodent—*Rattus jalorensis.*

Gahrliepia (Walchia) soricicola Traub & Evans

Gahrliepia (*Walchia*) *soricicola* Traub & E., 1957: ... (D).

Records. INDIA, North-east. Rodents—*Rattus r. yunnanensis.* Insectivores—*Anourosorex s. squamipes, Crocidura* sp. BURMA, North. Rodent—*Rattus r. sladeni.*

Gahrliepia (Walchia) turmalis Gater

Gahrliepia (*Walchia*) *turmalis*, Wom. 1952: 288 (76) d (? Burma records excluded), Audy 1954*b*, 1956*d*: 100 (cd), Traub & E. 1957: ... (dtc), Wom. & A. 1957 (xl), Har. 1957 (b).

Walchia turmalis, Fuller 1952: 45 (dtc), W. & F. 1952: 93, IMR 1953: 56, Rad. 1954: 270.

Walchia (?) *turmalis*, Gun. 1952: 45.

Records. BURMA, North. Rodents—*Rattus r. sladeni.* Insectivores—*Suncus murinus fulvocinereus, Tupaia glis versurae.* CEYLON (requires confirmation). Insectivore—*Suncus murinus giganteus.* MALAYA. Rodents—*Rattus bowersi, R. edwardsi,* ? *R. mulleri, R. sabanus.*

Gahrliepia (Walchia) ventralis Womersley

Gahrliepia (*Walchia*) *brennani* var. *ventralis* Wom., 1952: 295 (112) D, *not* Jam. & Tosh. 1954: 12 (dc), Audy 1956*d*: 100 (cd).

Gahrliepia (*Walchia*) *ventralis*, Traub & E. 1957: ... (dtc), Wom. & A. 1957 (xl), Har. 1957 (b).

Records. MALAYA. Rodents—*Rattus bowersi.*

Subfamily LEEUWENHOEKIINAE*

Genus **Leeuwenhoekia** Oudemans, 1911

Type: *Heterothrombidium verduni*, Oudemans, 1910

Subgenus **Comatacarus** Ewing, 1942

Type: *Comatacarus americanus* Ewing, 1942

? Leeuwenhoekia (Comatacarus) atollensis† (Radford), new combination

Comatacarus atollensis Rad., 1954*a*: 237 (D), 1954: 269.

Records. INDIAN OCEAN, Maldive Is. Rodent—*Rattus r. alexandrinus* (? *R. norvegicus, err. det.*).

Genus **Acomatacarus** Ewing, 1942

Subgenus **Acomatacarus** Ewing, 1942

Type: *Acomatacarus arizonensis* Ewing, 1942

Acomatacarus (Acomatacarus) adelaideae (Womersley)

Acomatacarus (*Acomatacarus*) *adelaidae*, W. & F. 1952: 97.

Acomatacarus adelaidae, Gun. 1952: 40, Rad. 1954: 268.

* A tabular summary of characters of the Leeuwenhoekiinae and Apoloniinae was given by Audy 1954*b*: 165. Mr Womersley has completed a monograph on the Leeuwenhoekiidae describing a number of new species (personal communication).

† This species has a median projection of the scutum, not indicated by the author. Its leg-segmentation (7.7.7) would place it outside the Leeuwenhoekiinae as at present understood, but it is best left where it is pending clarification.

Acomatacarus (Acomatacarus) athertonensis Womersley

Acomatacarus (*Acomatacarus*) *athertonensis*, W. & F. 1952: 97.
Acomatacarus athertonensis, Gun. 1952: 40, Rad. 1954: 268.

Acomatacarus (Acomatacarus) attolus (Banks)

Acomatacarus (*Acomatacarus*) *attolus*, W. & F. 1952: 97.
Acomatacarus attolus, Gun. 1952: 41.

Acomatacarus (Acomatacarus) audyi Radford

Acomatacarus (*Acomatacarus*) *audyi*, W. & F. 1952: 97.
Acomatacarus audyi, Gun. 1952: 41, Audy *et al.*, 1953: 36 (c), Rad. 1954: 269.

Acomatacarus (Acomatacarus) australiensis (Hirst)

Acomatacarus (*Acomatacarus*) *australiensis*, W. & F. 1952: 97.
Acomatacarus australiensis, Gun. 1952: 39, Rad. 1954: 268.

Acomatacarus (Acomatacarus) barrinensis Womersley

Acomatacarus (*Acomatacarus*) *barrinensis*, W. & F. 1952: 98.
Acomatacarus barrinensis, Gun. 1952: 41, Rad. 1954: 269.

Acomatacarus (Acomatacarus) dromus (Womersley)

Acomatacarus (*Acomatacarus*) *dromus*, W. & F. 1952: 98.
Acomatacarus dromus, Gun. 1952: 41.

Acomatacarus (Acomatacarus) echidnus Womersley

Acomatacarus (*Acomatacarus*) *echidnus*, W. & F. 1952: 98.
Acomatacarus echidnus, Gun. 1952: 41, Rad. 1954: 269.

Acomatacarus (Acomatacarus) ? gymnodactyla (Ewing)

Acomatacarus (*Acomatacarus*) ? *gymnodactyla*, W. & F. 1952: 98.
Odontacarus gymnodactyla, Rad. 1954: 268.

Acomatacarus (Acomatacarus) hirsti (Womersley)

Acomatacarus (*Acomatacarus*) *hirsti*, W. & F. 1952: 98.
Acomatacarus hirsti, Gun. 1952: 40, Rad. 1954: 269.

Acomatacarus (Acomatacarus) keegani Radford

Acomatacarus keegani Rad., 1953*b*: 242 (D), 1954: 269.
Records. PHILIPPINES. Bird—*Centropus melanops.*

Acomatacarus (Acomatacarus) longipes Womersley

Acomatacarus (*Acomatacarus*) *longipes*, W. & F. 1952: 99.
Acomatacarus longipes, Gun. 1952: 40, Rad. 1954: 269.

Acomatacarus (Acomatacarus) lygosomae Dumbleton

Acomatacarus (*Acomatacarus*) *lygosomae*, W. & F. 1952: 99.
Acomatacarus lygosomae, Gun. 1952: 41, Rad. 1954: 269.

Acomatacarus (Acomatacarus) mccullochi, Womersley

Acomatacarus (*Acomatacarus*) *mccullochi*, W. & F. 1952: 99.
Acomatacarus mccullochi, Gun. 1952: 40, Rad. 1954: 269.

Acomatacarus (Acomatacarus) nova-guinea (Womersley)

Acomatacarus (*Acomatacarus*) *nova-guinea*, W. & F. 1952: 99.
Acomatacarus nova-guinea, Gun. 1952: 40, Rad. 1954: 269.

Acomatacarus (Acomatacarus) patrius Womersley

Acomatacarus (*Acomatacarus*) *patrius*, W. & F. 1952: 100.
Acomatacarus patrius, Gun. 1952: 41, Rad. 1954: 269.

Acomatacarus (Acomatacarus) retentus (Banks)

Acomatacarus (*Acomatacarus*) *retentus*, W. & F. 1952: 100.
Acomatacarus retentus, Gun. 1952: 41, Rad. 1952: 269.

Acomatacarus (Acomatacarus) romeri Womersley

Acomatacarus romeri Wom., 1957*b*: ... (D).
Records. Hong Kong. Rodents—*Rattus r. flavipectus, R. r. sladeni, R. rattus* subsp.

Acomatacarus (Acomatacarus) southcotti (Womersley)

Acomatacarus (*Acomatacarus*) *southcotti*, W. & F. 1952: 100.
Acomatacarus southcotti, Gun. 1952: 40, Rad. 1954: 269.

Genus **Hannemania** Oudemansi 1911

Type: *Heterothrombidium hylodeus* Oudemans, 1910

Hannemania rouxi Oudemans

Hannemania rouxi, W. & F. 1952: 103, Rad. 1954: 268.

Genus **Whartonia** Ewing, 1944

Type: *Hannemania nudosetosa* Wharton, 1938

Whartonia penthetor Womersley

Hannemania sp.nov. Wom. in MS, Audy 1952: 151 (c).
Whartonia 'PEN', Audy 1956*d*: 100 (ct).
Whartonia penthetor, Wom. 1957*a*: ... (D).
Records. Malaya. Bats—*Cynopterus* (*Penthetor*) *lucasi*.

Whartonia vellae (Dumbleton)

Whartonia vellae, W. & F. 1952: 104.
Hannemania vellae, Gun. 1952: 39, Rad. 1954: 268.

FAUNAL LIST

This list contains only records which require to be added to the Faunal List of Wharton & Fuller (1952: 108–14). The arrangement follows that of Wharton & Fuller's List with the following exceptions: India and Pakistan are grouped together, the letters E, N, and P being used to indicate the main collecting regions; Hong Kong is added to the list, under Asia; and the genera of chiggers are listed alphabetically because of the number of changes which have taken place since the publication of the list on which the present one is based.

ASIA

Burma

Ascoschongastia (*Laurentella*) *audyi*, *A.* (*L.*) *soekaboemiensis*, *Gahrliepia* (*Gahrliepia*) *darita*, *G.* (*G.*) *evansi*, *G.* (*G.*) *fimbriata*, *G.* (*G.*) *gemina*, *G.* (*G.*) *tenella*, *G.* (*Schongastiella*) *erula*, *G.* (*S.*) *helata*, *G.* (*S.*) *ligula*, *G.* (*S.*) *punctata*, *G.* (*Walchia*) *disparunguis disparunguis*, *G.* (*W.*) *enode* (?), *G.* (*W.*) *erana*, *G.* (*W.*) *ewingi ewingi*, *G.* (*W.*) *morobensis*, *G.* (*W.*) *rustica*, *G.* (*W.*) *soricicola*, *G.* (*W.*) *turmalis*, *Trombicula* (*Leptotrombidium*) *lanceolata*, *T.* (*L.*) *longiseta*.

Hong Kong

Acomatacarus (*Acomatacarus*) *romeri*, *Ascoschongastia* (*Laurentella*) *indica*, *Helenicula comata*, *H. globulare* (?), *H. hongkongensis*, *H. lanius*, *Gahrliepia* (*Gahrliepia*) *romeri*, *G.* (*Walchia*) *turmalis*, *G.* (*W.*) *ventralis*, *Trombicula* (*Leptotrombidium deliensis*.

India and Pakistan

(E = North-east (Manipur and Assam); N = North (Kashmir, Kumaon);
P = the rest of peninsular India and Pakistan.)

Ascoschongastia (*Ascoschongastia*) *capillata* P, *Euschongastia* (*Walchiella*) *lewthwaitei* E, *E. schlugeri* P, *Gahrliepia* (*Gahrliepia*) *barbigera* N, *G.* (*G.*) *darita* E, *G.* (*G.*) *dupliseta* E, *G.* (*G.*) *plurisetae* E, *G.* (*Schongastiella*) *ceylonica* N, *G.* (*S.*) *gammonsi* E, *G.* (*S.*) *kalrata* N, *G.* (*S.*) *kumaonensis* N, *G.* (*S.*) *liota* E, *G.* (*Walchia*) *soricicola* E, *Helenicula comata* N, *Neotrombicula anax* N, *N. kanzalwanensis* N, *N. kashmirensis* N, *Schongastia pseudoschuffneri* E, *Schoutedenichia jubbulporensis* P, *S. nausheraensis* N, *Trombicula* (*Leptotrombidium*) *bhimtalensis* N, *T.* (*L.*) *dux* N, *T.* (*L.*) *fulleri* E, *T.* (*L.*) *macacus* E, *T.* (*L.*) *parapalpalis* N, *T.* (*L.*) *pelta* N, *T.* (*L.*) *puta* N, *T.* (*L.*) *radfordi* E, *T.* (*L.*) *roonwali* E, *T.* (*L.*) *tithwalensis* N, *T.* (*L.*) *villosa* N, *T.* (*Miyatrombicula*) *buxtoni* N, *T.* (*M.*) *rajoriensis* N, *T.* (*Trombicula*) *khurdangensis* N, *T.* (*Trombiculindus*) *cuneatus* E, *T.* (*T'us*) *foliaceus* E, *T.* (*T'us*) *fordi* E, *T.* (*T'us*) *plumosa* E, *T.* (*T'us*) *traubi* E, *T. vietzi* N; *G.* (*Walchia*) *manipurensis*.

Malaya

Ascoschongastia (*Laurentella*) *audyi*, *A.* (*L.*) *sellnicki*, *Babiangia booliati*, *B. parmifera*, *Euschongastia* (*Walchiella*) *nadchatrami*, *E.* (*Walchiella*) *traubi*, '*E.*' *lipoxena*, *Eutrombicula* (*Eltonella*) *eltoni*, *E.* (*Elt.*) *tweediei*, *Eut. fieldi*, *Fonsecia celesteae*, *Gahrliepia* (*Gahrliepia*) *neterella*, *G.* (*G.*) *ornata*, *G.* (*G.*) *picta*, *G.* (*G.*) *tessellata*, *G.* (*Schongastiella*) *argalea*, *G.* (*S.*) *arona*, *G.* (*S.*) *birella*, *G.* (*S.*) *hipposideros*, *G.* (*Walchia*) *brennani*, *G.* (*W.*) *cuspidata*, *G.* (*W.*) *disparunguis pingue*, *G.* (*W.*) *ventralis*, *Pseudoschongastia novita*, *Schoutedenichia vercammeni*, *Siseca subrara*, *Traubacarus alticola*, *Traub. brachypus*, *Traub. browningi*, *Traub. domrowi*, *Traub. harrisoni*, *Traub. intermedia*, *Traub. jadini*, *Traub. owenevansi*, *Traub. varmai*, *Traub. vercammeni*, *Trombicula* (*Leptotrombidium*) *langati*, *T.* (*L.*) *muridia*, *T.* (*Vorcana*) ? *vorca*, *T. consueta*, '*T*' *dimolinae*, '*T*' *harrisoni*, *T. leveri* (?), *T. revelae*, *T. taphozous*, *Whartonia penthetor*.

Thailand

Ascoschongastia (*Laurentella*) *audyi*, *A.* (*L.*) *indica*, *Gahrliepia* (*Gahrliepia*) *elbeli*, *G.* (*G.*) *fenestrulata*, *G.* (*G.*) *marshi*, *G.* (*G.*) *tylana*, *G.* (*Walchia*) *ewingi lupella*, *G.* (*W.*) *kritochaeta*, *G.* (*W.*) *micropelta*, *G.* (*W.*) *rustica*, *Trombicula* (*Leptotrombidium*) *deliensis*.

Australia

Ascoschongastia (?) *raui*, *Babiangia bulbifera*, *Euschongastia* (*Derrickiella*) *peregrina*, *E.* (*D.*) *wongabelensis*, *E. andromeda*, *E. dumosa*, *E. lappacea* (? syn.= *cassiope*), *E. mackerrasae*, *E. parva*, *E. popei*, ?*E.* [?*Trombiculindus*] *pseudomys*, *Eutrombicula* (*Eutrombicula*) *ablephara* (?), *Eutr.* (*E.*) *tovelli*, *Eutr. incurva*, *Eutr. lygosomoides*, *Neoschongastia entomyza*, *N. rectangulare*, *N. struthidia*, *Neotrombicula antechinus*, *Neotr. derricki*, *Neotr. mackayensis*, *Neotr. thylogale*, *Schongastia* (?) *bidentata*, *Siseca lundbladi*, *Sis. southcotti*, *Sis. thori*, *Trombicula* (*Leptotrombidium*) *myzantha*, *T.* (?) *geckobia*, *T.* (?) *gymnodactyla*, *T.* (?) *pentagona*, *T. southcotti*, *T. victoriensis*.

Islands of Indian Ocean

Ceylon

Ascoschongastia (?) *oculicola*, *Gahrliepia* (*Schongastiella*) *ceylonica*, *G.* (*S.*) *punctata*, *G.* (*Walchia*) *disparunguis pingue* (?), *G.* (*W.*) *turmalis*, *Trombicula* (*Leptrombidium*) *jayewickremei*, *T.* (*Trombicula*) *rrunda*, *T.* (*T.*) *spicea*.

Indonesia (plus Sarawak and British North Borneo)

Ascoschongastia (*Laurentella*) *audyi*, *A.* (*L.*) *daria*, *A.* (*L.*) *indica*, *A.* (*L.*) *indicella*, *A.* (*L.*) *labuanensis*, *A.* (*L.*) *ocellifera*, *A.* (*L.*) *roluis*, *Euschongastia* (*Walchiella*) *asonluca*, *E.* (*W.*) *calunosa*, *E.* (*W.*) *sarawakensis*, *Helenicula signata*, *Gahrliepia* (*Gahrliepia*) *granulata*, *G.* (*G.*) *laciniata*, *G.* (*G.*) *penetrans*, *G.* (*G.*) *tuberculata*, *G.* (*Walchia*) *ewingi ewingi*, *G.* (*W.*) *morrowae*, *G.* (*W.*) *naniparma*, *Traubacarus domrowi*, *Trombicula* (*Leptotrombidium*) *akamushi*, *T.* (*L.*) *baluensis*, *T.* (*L.*) *deliensis*, *T.* (*L.*) *micula*, *T.* (*L.*) *pilalta*, *T.* (*L.*) *pipellae*, *T.* (*L.*) *tarsala*, *T.* (*Vorcana*) *vorca*.

Celebes

Gahrliepia (*Walchia*) *disparunguis* (?), *Trombicula* (*Vorcana*) *densipiliata* ? subsp.

Java

Gahrliepia (*Walchia*) *ewingi ewingi* (?), *Schongastia pseudoschuffneri*.

ISLANDS OF PACIFIC OCEAN

New Guinea

Euschongastia crinita, E. innisfailensis, E. mohri, Eutrombicula (*Eutrombicula*) *ablephara, Eut.* (*E.*) *sobrina, Neoschongastia owiensis, Radfordiana rostrata, Schongastia whartoni, Trombicula* (*Leptotrombidium*) *myzantha, T. philipi*.

Philippines

Acomatacarus (*Acomatacarus*) *keegani, Helenicula comata, Trombicula* (*Vorcana*) *samara*.

Samoa

Schongastia (?) *samoaensis*.

Solomon Islands

Neoschongastia mcqueeni, Trombicula (*Vorcana*) *densipiliata dumbletoni*.

SUMMARY

A checklist is given of the 320 species and four subspecies of trombiculid mites of the Oriental and Australasian regions described up to the end of 1955, together with a number from papers being published in 1956 and 1957 and consulted by the writer in manuscript or proofs.

Eight new genera, six subgenera, and 175 species from the Oriental and Australasian regions have been described since the compilation of the checklist of world trombiculids by Wharton & Fuller (1952).

The present list gives only the documentation and records necessary to bring the appropriate part of Wharton & Fuller's publication up to date. Cross-references are given to the monograph by Womersley (1952) and its appendix by Womersley & Audy (1957), as well as to the checklists of Gunther (1952) and Radford (1954).

The taxonomic arrangement has been revised in accordance with recent publications. Four new combinations of species of *Neotrombicula* are recorded.

A supplementary Faunal List is presented, confined to records which now require to be added to the Faunal List of Wharton & Fuller.

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MEHRACULA SINHA, 1954, A SYNONYM OF *LEPTOTROMBIDIUM* NAGAYO *ET AL.*, WITH CORRIGENDA

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In a previous paper (Sinha, 1954), dealing with a collection of larval trombiculids from Manipur, India, four new species, a new subgenus (*Plumosicola*), and a new genus (*Mehracula*) were described. The larvae concerned had been mounted in batches, each slide containing considerable numbers. At the request of Dr J. R. Audy, Institute for Medical Research, Kuala Lumpur, the type material was lent to him in 1956 through the kind co-operation of Dr A. P. Kapur, in charge of the Entomology Section, Zoological Survey of India, Calcutta, in whose care the types had been deposited, and who gave permission for the specimens to be remounted individually in PVA-mountant.

I am grateful to Dr Audy for his comments on the type material, and to his assistant Mr M. Nadchatram, for remounting the slides. As a result (*a*) a few corrigenda may be noted, (*b*) some remounted paratypes will be available for distribution, and (*c*) the genus *Mehracula* can be synonymized.

Mehracula Sinha, 1954 is a synonym of *Leptotrombidium* Nagayo *et al.* 1916, which includes the major vectors of scrub-typhus. The genus *Mehracula* was characterized by annulate body-markings (which are identical with body-striae) and a subapical cheliceral denticle (which accompanies the tricuspid cap and is also found in other groups). Comments on the species follow.

Trombicula (Leptotrombidium) roonwali (Sinha), new combination
Mehracula roonwali Sinha, 1954, p. 329

The 'palpal formula' (as employed by Audy) is N.N.BNB, not N.N.NBN as recorded—the barbed ventral tibial seta often *appears* to be lateral. The dorsal setae, numbering 40–44 are usually arranged 2.10.8.2.10.8.4. This species is extremely close to *T.* (*L.*) *macacus* Womersley, 1952, but has not been compared with the unique type. The holotype (no. R 2/14.11.45) and 54 paratypes have been remounted separately. They were accompanied by 5 *T.* (*L.*) *deliensis* Walch. The host species is now recognized as *Tupaia glis belangeri* (Wag.); 'Manipur-Taru Road' for the locality should read 'Manipur (Imphal)-Tamu Road'.

Trombicula (Leptotrombidium) radfordi (Sinha), new combination
Mehracula radfordi Sinha 1954, p. 330

The galeal seta, and the dorsal seta on the palpal tibia, are barbed (not nude). The dorsal body setae are arranged 2.10.10.2.8.(10).8.6.2. Slide R 2/1.12.45 has been remounted.

Walchia manipurensis Sinha 1954, p. 335, is being redescribed by Traub & Evans (1957) who place it in *Gahrliepia* Ouds. *s.l.* (subgenus *Walchia*) after Womersley (1952).

The particular collection to which this paper refers was made in the same collecting area described by Audy, Thomas & Harrison (1953).

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